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## CONTENTS

### No. 1. FEBRUARY 1, 1920

STUDIES ON THE CEREBROSPINAL FLUID. <i>Frank C. Becht</i> .....	1
STUDIES ON THE CEREBROSPINAL FLUID. <i>F. C. Becht and P. M. Matill</i> .....	126
PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.....	174

### No. 2. MARCH 1, 1920

BLOOD VOLUME STUDIES. I. EXPERIMENTAL CONTROL OF A DYE BLOOD VOLUME METHOD. <i>C. W. Hooper, H. P. Smith, A. E. Bell and G. H. Whipple</i> .....	205
BLOOD VOLUME STUDIES. II. REPEATED DETERMINATION OF BLOOD VOLUME AT SHORT INTERVALS BY MEANS OF THE DYE METHOD. <i>H. P. Smith</i> ...	221
BLOOD VOLUME STUDIES. III. BEHAVIOR OF LARGE SERIES OF DYES INTRODUCED INTO THE CIRCULATING BLOOD. <i>A. B. Dawson, H. M. Evans and G. H. Whipple</i> .....	232
BLOOD VOLUME STUDIES. IV. BLOOD VOLUME AS DETERMINED BY THE CHANGE IN REFRACTIVITY OF THE SERUM NON-PROTEIN FRACTION AFTER INJECTION OF CERTAIN COLLOIDS INTO THE CIRCULATION. <i>Irvine McQuarrie and Nelson C. Davis</i> .....	257
THE INFLUENCE OF SPLENIC EXTRACT ON THE NUMBER OF CORPUSCLES IN THE CIRCULATING BLOOD. <i>Ardrey W. Downs and Nathan B. Eddy</i> .....	279
PHYSIOLOGIC CHANGES PRODUCED BY VARIATIONS IN LUNG DISTENTION. II. EFFICIENCY OF THE PULMONARY CIRCULATION IN OVERCOMING OBSTRUCTION. <i>Felix P. Chillingworth and Ralph Hopkins</i> .....	289
EXPERIMENTS ON THE PATHOLOGICAL PHYSIOLOGY OF ACUTE PHOSGENE POISONING. <i>Walter J. Meek and J. A. E. Eyster</i> .....	303
TO WHAT EXTENT ARE THE PHYSIOLOGICAL EFFECTS OF CARBON DIOXIDE DUE TO HYDROGEN IONS? <i>M. H. Jacobs</i> .....	321
THE GASTRIC RESPONSE TO FOODS. VII. THE RESPONSES OF THE NORMAL HUMAN STOMACH TO VEGETABLES PREPARED IN DIFFERENT WAYS. <i>Raymond J. Miller, Harry L. Fowler, Olaf Bergeim, Martin E. Rehfuess and Philip B. Hawk</i> .....	332
FURTHER OBSERVATIONS ON THE RELATION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPERGLYCEMIAS (ETHER AND ASPHYXIA). <i>G. N. Stewart and J. M. Rogoff</i> .....	366
THE EXPERIMENTAL PRODUCTION OF EDEMA AS RELATED TO PROTEIN DEFICIENCY. <i>Emma A. Kohman</i> .....	378

No. 3. APRIL 1, 1920

THE EFFECT OF THE SUBCUTANEOUS INJECTION OF ADRENALIN CHLORID ON THE HEAT PRODUCTION, BLOOD PRESSURE AND PULSE RATE IN MAN. <i>Irene Sandiford</i> .....	407
THE APPARENT INFLUENCE OF A DIET OF CARBOHYDRATES ON THE PANCREAS REMNANT OF PARTIALLY PANCREATECTOMIZED DOGS. <i>V. W. Jensen and A. J. Carlson</i> .....	423
THE COMPARATIVE PERFORMANCE OF MUSCLES SUBJECTED TO RHYTHMIC AND ARRHYTHMIC STIMULATION. <i>Harold A. Bulger and Percy G. Stiles</i> .....	430
RENAL ACTIVITY AND THE ACID BASE EQUILIBRIUM. <i>T. Nagayama</i> .....	434
THE UREA EXCRETING ACTIVITY OF THE KIDNEY AND PHOSPHATE EXCRETION. <i>T. Nagayama</i> .....	449
GASTRIN STUDIES. III. THE RESPONSE OF THE STOMACH MUCOSA OF VARIOUS ANIMALS TO GASTRIN BODIES. <i>R. W. Keeton, F. C. Koch and A. B. Luckhardt</i> .....	454
GASTRIN STUDIES. IV. THE RESPONSE OF THE STOMACH MUCOSA TO FOOD AND GASTRIN BODIES AS INFLUENCED BY ATROPINE. <i>R. W. Keeton, A. B. Luckhardt and F. C. Koch</i> .....	469
FURTHER OBSERVATIONS ON THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS, AND, THE ACTION OF STRYCHNINE AFTER CERVICAL CORD SECTION. <i>G. N. Stewart and J. M. Rogoff</i> .....	484
THE RELATION OF CATALASE TO HEART ACTIVITY. <i>R. J. Seymour</i> .....	525
THE EFFECT OF VITAMINE DEFICIENCY ON VARIOUS SPECIES OF ANIMALS. I. THE PRODUCTION OF XEROPHTHALMIA IN THE RABBIT. <i>Victor E. Nelson and Alvin R. Lamb</i> .....	530
THE FLASHING INTERVAL OF FIREFLIES—ITS TEMPERATURE COEFFICIENT—AN EXPLANATION OF SYNCHRONOUS FLASHING. <i>Charles D. and Aleida v.'t H. Snyder</i> .....	536
EFFECT OF DIMINISHED OXYGEN UPON RATE OF NERVE CONDUCTION IN CASSIOPEA. <i>Alfred Goldsborough Mayor</i> .....	543
THE ALKALI RESERVE OF THE BLOOD PLASMA, SPINAL FLUID AND LYMPH. <i>J. B. Collip and P. L. Backus</i> .....	551
THE EFFECT OF PROLONGED HYPERPNOEA ON THE CARBON DIOXIDE COMBINING POWER OF THE PLASMA, THE CARBON DIOXIDE TENSION OF ALVEOLAR AIR AND THE EXCRETION OF ACID AND BASIC PHOSPHATE AND AMMONIA BY THE KIDNEY. <i>J. B. Collip and P. L. Backus</i> .....	568
IS THE LUMINESCENCE OF CYPRIDINA AN OXIDATION? <i>E. Newton Harvey</i> ....	580
A PHYSIOLOGICAL RESPONSE TO PITUITARY ADMINISTRATION. <i>F. S. Hammett, C. A. Patten and N. Suitsu</i> .....	588
INDEX.....	593

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## CONTENTS

	PAGE
STUDIES ON THE CEREBROSPINAL FLUID. <i>Frank C. Becht</i> .....	1
STUDIES ON THE CEREBROSPINAL FLUID. <i>F. C. Becht and P. M. Matill</i> .....	126
PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY .....	174

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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## STUDIES ON THE CEREBROSPINAL FLUID

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Received for publication August 27, 1919

### I. A CRITICAL STUDY OF THE METHODS EMPLOYED IN THE OBSERVATIONS ON THE CEREBROSPINAL FLUID

#### *Introduction*

Recent publications regarding the formation of the cerebrospinal fluid have been so numerous and at first glance so complete and carefully controlled, that further work on the subject should be unnecessary. To many this seems to constitute a completed chapter in physiology, for they believe that it has been established definitely and clearly that the cerebrospinal fluid is formed by a secretory process, and that this process is capable of stimulation and inhibition by substances of plant and animal origin. However, the fact that some important mechanical factors have been disregarded by some and under-estimated by others, justifies us in the publication of a paper embodying the results of our experiments. We wish to introduce in considerable detail the data from our work—a procedure followed by none of our recent predecessors in the field—so the reader may satisfy himself in regard to the number of experiments, the results, and the variations between experiments where variations existed.

#### *General statement of the problem*

An analysis of the problem involved and a review of the literature covering the formation of the cerebrospinal fluid, show that the subject divides naturally into two sections: The study of the actual separa-

tion of the fluid from the blood or lymph, and the laws governing the process; and the study of the movement of the fluid lying free in the sub-arachnoid space and its communicating cavities. This latter study is a complex one, because the fluid may or may not be retained in the various recesses of the subdural spaces and the central canal when the attempt is made to drain out the pre-formed fluid, or to collect that newly formed. Besides, to that already present more may be added by new formation, or from it some may be removed by absorption along the natural channels. Writers on this subject have not discriminated with sufficient care between these two phases. Many may have been studying the movements of the fluid while assuming that they were studying the formation of the fluid. This, together with the fact that conclusions are sometimes given instead of experimental data, and that no mention is made of the number of experiments or of the limits of variations between experiments, leaves the reader much in doubt regarding the truth of the situation in the study of the formation of the cerebrospinal fluid.

*Critical review of the status of the formation of cerebrospinal fluid*

The first problem—the study of the actual separation of the fluid and the mechanism involved—has never been solved sufficiently to warrant a definite statement regarding even the structure concerned in the formation. Thus the mechanism of the separation must of necessity have remained untouched. The general consensus of opinion regarding the process is that it is a secretory one, an idea advocated first by Faivre in 1853 (1). The secretory theory was held to be supported by the observation made by Cappelletti (2) that pilocarpine and other drugs usually considered secretogogues increased the amount of fluid which escaped from a temporary or permanent fistula made into the dural canal. The direct evidence that the process is secretory, and that the choroid plexus is concerned in the process is purely anatomical. Petit and Girard (3) described changes in the cells of the choroid plexus after secretory drugs, particularly after pilocarpine and muscarine, changes which were interpreted as evidences of secretion. This work was confirmed and extended by Meek (4). To quote from the latter author:

In the case of the rabbit and the guinea pig muscarine produces decisive results.



Often as many as two-thirds of the cells show evidence of secretion. Normally the epithelial cells in the rabbit are six micra high, but here the height has increased to twelve micra. A differentiation into two zones, a basal granular and an outer clear zone is suggested, but is not so well marked as in the figures of Petit and Girard. The granulation, however, is always heavier and more compact toward the base of the cell. Clear spaces begin to appear toward the top, and rarely does the stainable cytoplasm extend to the upper cell wall. Masses of larger granules are common in the upper part of the cell, where the lines forming the reticulations cross. The nucleus remains globular with a clear outline, in fact it is not distinguishable from that of the resting cell. The two things most striking about these modified cells are their increase in height and the appearance of so much clear space at the distal side of the nucleus.

If we turn now to the work of Langley (5), we find the following (p. 263) regarding the parotid gland of the rabbit:

The alveoli of the parotid of a hungry rabbit taken fresh from the animal and at once examined are granular throughout; there is no division into an outer clear and an inner granular zone. . . . But if the gland be thrown for some time into a state of activity either by stimulating the sympathetic in the neck, or by injecting pilocarpine, or by feeding, the alveoli alter their appearance and instead of being granular throughout become clear at their outer portion near the basement membrane, and thus show an inner granular and an outer clear, non-granular, zone. . . . The longer the gland is stimulated the more obvious the outer clear zone becomes, so that at length some alveoli may show scarcely any granules. . . . The cells, too, . . . become smaller . . . at the same time such nuclei as were irregular, or were compressed become spherical, and move somewhat toward the center of the cell . . . (p. 279). In the mucous glands during secretion the changes in life are less readily followed, but they are probably similar to those mentioned above. In activity the granules are used up, and disappear first from the peripheral parts of the cell. . . . The infraorbital and lachrymal glands show an outer clear and an inner granular zone in activity, even more distinctly than the parotid.

A comparison of these statements leads one to suspect some error. In the case of the parotid and other glands examined by Langley, resting cells are granular throughout, and during activity become *smaller*, the cytoplasm is differentiated into an *inner* (i.e., toward the free edge) *granular* and an *outer* (basal) *clear* zone, and the *nuclei become less distorted* and move toward the center of the cell. In the case of the choroid plexus the resting cells are granular throughout; during activity the cells become *larger*—at least they are doubled in height, the cytoplasm is differentiated into an *outer* (i.e., toward the free edge) *clear* and an *inner* (basal) *granular* zone and the *nucleus cannot be differentiated* from the nucleus of the resting cell. Here, then, are changes in two structures, changes, so far as can be determined from reading

the accounts, exactly opposite in character, yet both are considered evidences of physiological activity, and what is more puzzling as evidences of the same activity, viz., of secretion.

It is, therefore, evident that while anatomical changes are produced in the choroid plexus by the action of drugs commonly producing secretion, such as muscarine and pilocarpine, the significance of this change is one of interpretation only, and more than one interpretation is possible. Instead of considering that this change described in the choroid means secretion, one might with equal right assert that from the anatomical evidence the choroid plexus is an organ of absorption of the cerebrospinal fluid as shown by the increased height of the cells.

From the physiological side we have some experiments which bear on the general seat of formation of the fluid. Dandy and Blackfan (6) apparently were the first to produce artificial internal hydrocephalus. They occluded the aqueduct of Sylvius by introducing a gelatine capsule filled with cotton or gauze. The train of symptoms—lethargy and vomiting—dated from the time of operation, and was considered undoubted internal hydrocephalus. Frazier and Peet (7), using a slightly different method, observed the same phenomena in their animals, but the onset was noticeably slower. Neither of these authors specifically claim that their experiments prove that the fluid is formed by the choroid plexus, but it is easy for the reader to infer that the experiments make it more than probable that such is the case. In actual fact these experiments do not prove the point of origin of fluid beyond the fact that it may originate above the aqueduct of Sylvius; they simply confirmed the well-known facts of internal hydrocephalus. They do not prove the specific point of formation, for in the region under observation increased formation might result from activity of the choroid plexus, from stimulation of the ependyma cells lining the ventricles, from increased transudation from the capillaries or from the formation of intracranial lymph: any one or all of these factors may be involved. Further, the rate of formation may be perfectly normal, the whole pathology being merely decreased absorption.

The same objections hold for the experiments of Weed and Cushing (8), who in the title of their paper: "Studies on cerebrospinal fluid. The effect of pituitary extract upon its secretion (choroidorrhoe)" go on record as accepting as a fact the *secretion* of the fluid by the choroid plexus, and apply the term "choroidorrhoe" to the apparent but not necessarily real "increased secretory activity of the choroid."

These authors believe that the secretory rôle of the choroid in the formation of the fluid is proved by the work of Cappelletti (2), Petit and Girard (3) and others; and on the basis of secretion they explain their observations which are recorded in two places: *a*, Experiments described in general terms by Weed (9), in which the aqueduct was catheterized after which a few drops of fluid escaped. This was followed by a "flow for from three to four hours, the rate of escape from the catheter being gradually slowed. The final cessation of flow is apparently due to exhaustion of the choroid plexus under experimental conditions." *b*, Experiments reported by Weed and Cushing (8) on animals in which the fluid was allowed to escape from the *cisterna magna* and from the third ventricle. In some of the latter the aqueduct was catheterized, in others a needle was inserted into the third ventricle through the *corpus callosum*. Neither of these experiments proves the seat of origin of the fluid to be the choroid for any of the factors mentioned above may be concerned here as well. They do not eliminate positively in all their experiments the possibility of the flow being due entirely to preformed fluid, or to fluid formed under decreased pressure during the experiment. The details of the criticism will be taken up later. The observation of Cushing in man that ligation of the choroid is followed by a cessation of the secretion is to the point. Unfortunately the author does not state whether the immediate cessation was a stopping of the "sweating" process observed at the time of operation or whether the ligation was followed by a permanent cessation of symptoms due to excessive formation. We are inclined to believe that no excessive formation was present in the case cited by the author named, so the former must have been meant, for he says: "On one or two occasions, I have had the opportunity in man to observe the main plexus at the bottom of a large porencephalic cavity, emptied of its contents, and have seen the fluid exuding from the surface of the structure. In one . . . . the plexus was removed, and in another the entering blood vessels were ligated with immediate cessation of the secretion." He evidently considers this a direct observation of the *normal* function of the plexus in secreting the fluid. Weed observed sweating of the pia-arachnoid but considers it *pathological*, primarily due to alteration of the pressure on the membrane after removal of the calvarium. However, this observation was made long before that recorded by Weed, for Spina (10) reports: If adrenal extract is injected intravenously into a curarized dog, the brain which has been laid bare for direct observation becomes hyperemic, swells

and becomes more wet; or,—a fact upon which special emphasis must be placed—drops of fluid appear upon the surface during the time the brain is enlarging. Thus it becomes evident that the appearance of drops of fluid upon the appendages of the brain is not confined to one structure, the choroid, for it is seen over the whole surface; and thus it is impossible to decide from direct observation which one of these *normally* gives rise to the fluid. In the observations cited, those by Cushing were made under conditions practically identical with those by Weed; if one set was pathological, then the other must have been also. Neither does the effect of the ligation of the vessels entering the choroid plexus give convincing evidence on the subject, for such an operation would, by producing anemia, prevent either normal or pathological processes of new formation in almost any gland except the most resistant, and even in these activity would be possible only for a few minutes at most.

Experiments in dogs in which the choroid has been removed, should prove this point, but to our knowledge such experiments of a conclusive character, although attempted many times (24), never were performed with complete success, undoubtedly on account of the difficulty of removal of the plexus in the living animal. The reports in the literature are not complete enough, nor have enough experiments been performed successfully to draw definite conclusions as regard the function of the choroid, and its relation to fluid formation.

Finally, the experiments of Dixon and Halliburton (11), (12) may be interpreted as showing the specific action of the choroid in the formation of cerebrospinal fluid, for these authors claim to have observed increased formation of the fluid following the injection of extracts of the choroid plexus, homologous and heterologous in origin. The evidence offered on this point by these authors is not conclusive, because their results will be shown to be capable of an explanation on an entirely mechanical basis. These extracts produced marked mechanical changes in the vascular system, and these changes, not increased secretion in response to a specific hormone, may well have been the cause of the change in volume of the contents of the dural canal and hence of the fluid pressure, or the rate at which the fluid escaped from the needle. Further, the extracts of choroid plexus act upon other structures as well, having a rather marked lymphagogue action.

A consideration of the facts as stated shows clearly that the point of origin of the fluid, the structures concerned in its elaboration, and the nature of the process involved, are not known. Our own work in the

field does not bear on this point more than that of our predecessors. For that reason we say frankly that we do not know the point of origin nor the mechanism of formation of the cerebrospinal fluid. While far from being prejudiced against the secretory theory of the formation of the fluid, we do not believe that there is as yet any clear-cut evidence that the actual process of formation is secretory or that the fluid comes normally from the choroid plexus.

*Critical review of the movements of the fluid*

The second problem—the movements of the fluid in the canal—is the one usually studied when the problem of “the formation of the cerebrospinal fluid” is under consideration. But as has already been pointed out, the rate of outflow or the alterations in pressure may have absolutely no relation to the rate of new formation. The amount of fluid in the canal may remain constant, but owing to increased volume or pressure of blood in the skull, or the increased oscillations, the amount of fluid escaping or the pressure recorded by a manometer, may be markedly increased. The difficulties encountered in the study of this process are numerous: New formation and absorption may or may not be going on, both at unknown rates. If the method adopted requires the emptying of the dural canal, as so many do, some fluid will always be left behind, no one knows how much or how little. If a part or all of the fluid is emptied from the canal, the removal by reduction of pressure alters conditions within the system to such an extent that the mechanism of formation may change entirely, the normal being held in check and a new one instituted. The study of the cerebrospinal fluid formation is surrounded by the same difficulties encountered in the study of the formation of urine, if the experimenter had to work on an animal with a rigid abdomen, which could not be opened, with the bladder replaced by a rigid vessel of unknown size partly filled with unknown and varying amounts of fluid, some being newly formed, some being absorbed through the natural pathway, with the urethra the only avenue of approach. Moreover to increase the difficulty in the case of the fluid no natural avenue of approach exists, so an artificial one must be made, usually by the insertion of a needle at some convenient point into the canal.

Limiting the study to the behavior of the fluid rendered accessible by inserting a needle or cannula into the subarachnoid space or one of its communicating cavities, a review of the methods employed in

the study of the problem becomes necessary. These methods fall naturally into two classes and a number of modifications, each having some advantages over the other. In one case the attempt is made to determine the amount of fluid formed *directly*, by measuring the rate of outflow of fluid in drops; in the other, to arrive at the amount formed *indirectly* by measuring the alterations in the pressure of the fluid. As is evident from this first and simplest statement, the outflow method can record only those cases in which there is an increase in the fluid, and hence it is only as applicable as a maximum manometer would be to all the phenomena of blood pressures. The pressure method is adapted to measure both increases and decreases in the volume of fluid. Thus in our judgment the latter method is far preferable to the former or any of its modifications.

#### *Outflow methods*

1. *Temporary fistulae.* Temporary and permanent fistulae have been established. The first we noted to have used the fistula method was Magendie (13). To enumerate all those who have used this method, some employing the lumbar region and others the simpler expedient of inserting the needle into the canal through the occipito-atlantal ligament, would involve unnecessary repetition. This method, or some of its modifications, is the one most commonly used in the past. The slow dropping of fluid from the needle after the first gush has ceased has been considered the normal rate of formation, for it has been argued that a drop of fluid must have been formed within the canal, else the drop just lost would not have made its escape. The drops have been recorded electrically on a smoked drum, thus making a graphic record of the experiment.

Some of the sources of error in the outflow method are so obvious as to be self-evident, others are more subtle: The *first* is the objection to the alterations of pressure. Much of the fluid already present in the canal, and much of that possibly being formed during the course of the experiment, is drained out, thus reducing the pressure in the canal which is normally positive to, or almost to, zero—a change which in itself will probably produce changes in the rate of formation, in either a positive or a negative direction, with the former the more probable. For this reason it is more than likely that the enormous quantities of fluid reported by clinicians as draining from the ear or the nose, in cases of congenital defect or after compound fracture of

the skull, represent not the normal rate of formation but the surprising powers of the structures producing the fluid to regenerate it after loss. On the other hand, this fluid may not be of normal composition at all, but a transudate from the blood exuding in drops because of the abnormal differences between the pressure in the blood vessels and the pressure in the dural canal. A process of this kind can best be observed by injecting adrenalin intravenously after removal of the calvarium, so that the pia mater may be observed directly. In this experiment drops of water (fluid) appear on the pia and to this process the term "sweating" has been applied (10). Whether this is the normal mechanism working at a higher rate than normal, or an abnormal mechanism, has not been determined, but we believe the latter view to be the more probable one.

Some of the later investigators recognized the validity of this objection. Thus Weed and Cushing (8) attempted to avoid the reduction of the intradural pressure by substituting for the wide needle a narrow one or a catheter calibrated to deliver fluid under a pressure estimated to be equal to that normally existing in the canal. They limited the maximum pressure at all times to the resistance of the catheter or needle, even in the animals subjected to pituitrin injections in which it can be shown by other methods the fluid pressure may rise far above the normal. Hence their method does not do what they claim for it: "Pressures approximating the normal were maintained within the ventricles." For while the resistance offered by the catheter or the needle may be the normal at the *beginning* for the animal without injection, it ceases to be the normal with the injection, for this resistance does not increase as the pressure in the skull increases, as would be the case in the intact canal. Therefore, the outflow may show not new formation, but increased outflow of fluid displaced by the increased intracranial pressure, due to vascular changes. Since the vascular pressure changes and the pressure of the cerebrospinal fluid remains the same, being limited to the resistance of the needle, it follows that the differences in the pressure of the fluid in the vessels and the fluid in the subarachnoid space is greater by this method as well as by the other. These pressure differences are somewhat smaller than by the ordinary outflow method, but are in the same direction, hence the conditions necessary for filtration or for transudation exist but in a smaller degree in this method as well as in the older ones. (See fig. 6 in the following article).

A further objection is the fact that by the outflow method the pressure of fluid in the canal is limited artificially at all times to the pressure required to force fluid through the needle, instead of being higher or lower depending upon the vascular condition of the skull. For this reason under the influence of hemodynamic drugs or under physiological manipulation, the differences between arterial and venous pressures on the one hand and fluid pressure on the other become abnormally great—a condition which does not arise under some of the other methods employed. This error is only a variation of the error due to reducing the pressure by emptying the canal.

The *second* objection to the outflow method is the inability of any worker to empty the canal completely. A consideration alone of the complicated system of passages and reservoirs is sufficient to bear out this statement. There is sure to be blocking of a part of the fluid at some strait in the system either within or without the central nervous system. Then, later, when the experiment is under way, and the cord and the medulla move with each respiration and each heart beat—uniform movement of the anesthetic is properly adjusted—this preformed fluid flows from a higher to a lower level from gravity, or from a cavity under higher pressure to the fourth ventricle where the pressure is limited as a maximum to the pressure required to force liquid through the needle. Then it escapes gradually and steadily to the outside, simulating a “normal rate” of secretion. A uniform rate of escape, it is true, but this is not necessarily a measure of the rate of formation, for it may be only a measure of the rate of escape of preformed fluid, or the rate at which it may be formed if its free escape is permitted. This outflow of preformed fluid, we believe, is the so-called “normal rate of formation” recorded by many authors. This flow is said not to persist after the fifth hour, even in an animal as large as a goat (11), where the blood pressure is still fairly high at the end of the experiment, a fact which makes our explanation that the “normal rate of formation” represents the leakage of preformed fluid all the more probable.

Numerous expedients have been adopted to avoid this error. The best was the one adopted by Dixon and Halliburton who abandoned the outflow for the manometer method. Weed and Cushing attempted to eliminate it by showing that in some experiments after ventricular catheterization the total amount of fluid collected was greater than that normally found in the ventricles. This expedient is hardly satisfactory because the amount found in the ventricles varies within such wide limits. In our opinion 3 cc. is a large but not abnormal amount



of fluid, for we have several times collected as much from the ventricles of cats in some work on immunity, where normal animals were used. The expedient of transcallosal puncture did not avoid this error for when they enter the third ventricle by the transcallosal route, they leave the aqueduct of Sylvius open; therefore, as the pressure in the third ventricle is lowered by drainage all the fluid in the fourth ventricle and in the subarachnoid space can be drawn upon to make good the deficit. While it is believed the direction of the flow of fluid is normally from the third ventricle into the fourth through the aqueduct, there is no reason for thinking that under the conditions of this experiment the movement of fluid might not be in the opposite direction, also. This is borne out by experiment: If a saline solution of methylene blue is injected in small amount—1 cc.—into the fourth ventricle of an animal with a trocar in the third ventricle according to the method of Weed and Cushing, the dye appears at once in the fluid from the third ventricle. Low pressure was used in the injection. This shows that the fluid can pass from the fourth to the third ventricle, and that in the experiments described, except in those in which catheterization of the ventricle was practised, the fluid secured from the third ventricle was subject to the same influences as the fluid secured from the *cisterna magna* by the other method. And even in those experiments in which the third ventricle was catheterized the fluid is subject to the alteration of pressures and the data to the error pointed out for the outflow method in general. And in our opinion if time enough is given the subdural space will be drained by transcallosal puncture as well as by a puncture into the fourth ventricle. Closely related to this objection is the inability to differentiate between newly formed and preformed fluid.

Nearly all our recent predecessors (8), (11), (14), have depended upon the recession of fluid into the tube to differentiate between preformed and newly formed fluid. They believe that if there has been increased vascularity of the brain there will be an acceleration of the rate of flow (8, p. 101):

Then with subsequent lowering of the congestion on the arterial or venous side, as the case may be, the fluid will be observed to recede into the cannula to compensate for the decreasing vascularity. On the other hand, if fluid is actually being secreted no such recession is observed, and though the rate may be slowed fluid continues to appear in spite of the relatively devascularized brain.

We must point out that this method of differentiation need not give such good results as might at first glance be expected of it. It is well known that both brain and cord, because of their large water content, are practically incompressible; because of its bony structure the skull and neural canal are nearly undilatable to pressure except at the membranes covering the foramina between the vertebrae. To the same degree that the brain is incompressible and the skull undilatable by pressure are they lacking in elastic recoil when the pressure is removed. So when the vascular pressure falls or becomes stationary after the effects of the drug have worn off, no very efficient elastic rebound in the intracranial structures can occur, and hence no great negative pressure can be developed. For this reason the strong sucking back of fluid against the resistance offered by the catheter upon which they depended to differentiate the mechanical from the secretory need not occur at all. Further, the limitation of the intracranial pressure to the normal or less as a maximum, as is done in the outflow method, works against the development of a strong negative pressure. Only by exerting powerful pressure in compressing an object can that object respond by strong rebound when the pressure is removed. This constitutes a second reason why strong sucking back need not be a constant phenomenon in employing this method for the study of the cerebrospinal fluid. For the same reason it does not follow necessarily that in all those cases in which no recession in the tube was observed, new formation had occurred.

A *third* objection to the outflow method is the inability to judge the effect on the fluid of vascular change and readjustment. Alterations in the arterial and venous pressures in the skull exert more influence upon the fluid in the canal than a similar change in pressure in the blood vessels would upon fluid in any other part of the body. The brain is incompressible, the bony calvarium prevents any expansion, and thus any rise in arterial or in venous pressure exerts a marked effect upon the fluid pressure. The fluid then moves outward through the needle because of low pressure outside and high pressure within. Further, the cerebral arteries behave differently from other arteries of the body apparently becoming widened instead of narrowed under conditions of a general rise in blood pressure such as follow the administration of the drug adrenalin. At least the pressure in the subarachnoid space is markedly increased instead of decreased as would be expected if the cerebral vessels responded in a manner similar to blood vessels elsewhere. (See fig. 11). The explanation usually made, of

course, is that there are no vasomotor nerves to the vessels of the brain, or if such exist, they are so feeble in their action that the vessels are passively dilated under the influence of high general blood pressure in spite of the constriction attempted. The existence of vasomotor nerves to the brain has never been proved although many physiologists have carried out experiments in which their existence was suggested. Further, anatomists have described in the walls of the cerebral vessels, structures which in other parts of the body are considered vasomotor nerve endings. Under the conditions described, anything altering the arterial or venous pressure or the respiratory rate and depth will alter the rate of escape of the fluid. So long as the pressure is higher than normal, the fluid in the canal is under greater pressure, and some of it, which normally would remain in the canal, is forced out and drops off, a process which does *not necessarily cease when the pressure begins to fall*, but which may continue so long as the pressure is higher than it was in the beginning of the action. This persistence of the action is one of the factors which has led to confusion, but the explanation is easy if the true condition in the canal is recognized. This undoubtedly explains some of the flow of fluid persisting after arterial pressure has fallen. When the vascular normal is reestablished, the fluid which has dropped off is no longer available, and in some instances air is drawn into the cavity. This fact has been observed by nearly all the recent workers in the field. However, apparently they did not recognize that so far as that animal is concerned further observations are useless, for no one knows how much of the intracranial space is taken up by air, how much by fluid. Besides, with the outflow method as described the observer has no way of studying the effect of vascular readjustment upon the fluid for when the fluid has retreated out of sight into the tube, it does not reappear for a long time unless another hemodynamic drug is given. This mechanical forcing out of the fluid has been recorded as "secretion" and is the cause of the "increased formation" of fluid under the influence of hemodynamic drugs, to which class most of the drugs and extracts described as having a stimulating effect upon the formation of cerebrospinal fluid belong.

Thus the method is not applicable to a study of the fluid in those conditions in which a recession is to be expected. It is only as applicable to this problem as a maximum manometer is to the study of all the phenomena of blood pressure. When this method is used any rise in the arterial or venous pressure—produced by the pressor nature of the substance injected—raises the blood pressure in the skull sufficiently to force the fluid out through the catheter.

Frazier and Peet attempted to avoid this error by permitting the fluid to flow into a graduated tube adjusted level. In addition to having the point of outflow the lowest point in the system this method practically limits the observation to outflow only, for the very obvious reason that water never flows up hill, unless under the influence of some pressure. In an animal placed in the position they describe the regular pulsations to be observed in the brain and cord permit the fluid present to flow down the inclined planes offered by the cord and the meninges in one direction and by the brain in the other, and escape into the tube. This process is favored by a pressor effect upon the arterial and venous blood pressures, and by increased respiratory movements. As the effect of the drug or of the manipulation which induced the change in blood pressure wears off, and readjustment takes place within the skull, and the pressure having been limited throughout to the resistance of the needle as a maximum and thus low, the inelasticity of the structures under pressure prevents the development of enough negative pressure to suck the contents of the tube back, if there is much resistance from gravity, as would surely be the case in the experiments described. Since their records are in one-hundredths of a cubic centimeter, their tube must have been of fine bore, so to the force of gravity which has to be overcome must be added the resistance of the tube and capillarity. In spite of the marked disadvantage to which they subjected the fluid in their experiments, they observed return of the fluid in many cases. If a strong negative pressure could develop the increased formation might be due to "*edema ex vacuo*."

*Fourth*, it is conceivable that in an animal in which the outflow method is used, the resistance offered by the cerebrospinal fluid in passing through the needle outward from the skull is less than that offered by the blood in passing by natural channels to the heart. When this occurs the amount of blood accumulating in the sinuses would increase at the expense of the cerebrospinal fluid. The accumulation of blood would then displace an equal amount of fluid, thus setting up a pseudo-secretion without a rise either in cerebrospinal or in venous pressure.

*Fifth*, one further possibility must be considered: Under normal conditions found in the skull the transfer of fluid from the extracerebral part of the cerebral cavity to the cerebellar cavity and the fourth ventricle is more or less difficult, because of the fact that the way is almost completely blocked, mainly by the cerebral peduncles and the *tentorium cerebelli*. This was pointed out by Hill (18, p. 19), and we

would add that what space remains must be completely blocked by the circle of Willis and the sinuses at the base of the brain. Since this is true, it is easy to see that a rise in venous pressure will displace the fluid around the medulla and cerebellum readily with the outflow already described, but at the same time will render the passage of fluid from the cerebral cavity more difficult. However, Dixon and Halliburton have already shown that depressor substances like amyl nitrite increase the outflow of fluid. We will show that this is true, not only when venous pressure rises, but when it falls (fig. 36). Therefore, there must be furnished an adequate explanation of the mechanism of increased outflow with both arterial and venous pressures falling. We believe the correct explanation to be as follows: With a considerable amount of fluid present in the cerebral cavity, a fall in both arterial and venous pressure causes a shrinkage of the cranial contents, particularly of the sinuses around the base of the brain. This shrinkage increases the ease with which fluid passes this strait, and permits the contents of the extracerebral part of the cerebral cavity at a higher level than the medulla to flow down into the vicinity of the fourth ventricle and thus drain out through the needle. This explains many of the occasionally observed but by no means constant increased outflows with reduced arterial and venous pressures. If it so happens that the cerebral cavity is practically empty, then the shrinkage of these structures lets the fluid escape from the fourth ventricle into the cerebral cavity instead of through the needle with the result that fluid outflow decreases, stops altogether, or the fluid may recede in the needle. Both increased and decreased outflow with falling arterial and venous pressures have been observed. (See fig. 36).

The position adopted for their animals by Frazier and Peet (7, p. 275), "by placing the subject on an inclined plane head down so that the occipito-atlantal ligament was under the most dependent portion" would give the maximum effect so far as the outflow of fluid is concerned when substances reducing both arterial and venous pressures are injected. Their responses to tissue extracts were much more marked than our own using the outflow method.

*Sixth*, if the outflow method of recording the fluid is used it is absolutely necessary that respiratory tracings be taken. Increasing the respiratory movements increases the oscillation of fluid in the canal. Thus any drug or physiological manipulation which produces altered respiratory rhythm, irrespective of whether it alters blood pressure or not will have an influence upon the rate of flow from the needle. The

reason for this increased outflow is apparent. When the needle is inserted the canal is drained until the surface of the fluid in the canal is level with the lumen of the trocar. Then any increase in the oscillations of fluid in the canal such as would be produced by greater respiratory efforts will increase the leakage through the needle, in exactly the same way that waves produced on the surface of a tank would increase the leakage through a hole in the side at the level of the surface of the water. In addition the rate at which fluid will be released from the various cavities at higher levels or under greater pressure will be increased by the increased movements of the nervous system. On the other hand we have seen cases in which increasing the respiratory movement decreased the outflow of fluid, an observation to be explained on physical grounds only by the supposition that the fluid flows into some lower lying cavity, or into one under less pressure. (See fig. 17).

From this discussion it is clear that the outflow method for the study of the rate of formation of the cerebrospinal fluid is unsatisfactory.

To summarize the objections to the outflow method: The reduction of pressure within the dural canal, the inability completely to empty the canal, the limitation of the pressure to the resistance of the needle as a maximum, the inability of studying the behavior of the fluid during vascular readjustment by furnishing fluid to the canal while the adjustment is occurring, the inability to control changes due to alterations in respiratory rate and strength, all these make this method unsuitable for the problem. Dixon and Halliburton (11) used it in their early work, but discarded it later. We had already discarded it before the appearance of their papers. Using this method we secured graphs identical with theirs, but since so many factors were left uncontrolled, we discarded the experiments as worthless. Many recent writers have used this method without any recognition of its limitations. We believe that to prove normal new formation by the outflow method, it must first be granted that complete emptying of the canal is possible, and that the reduction of the pressure does not alter the processes of formation either as regards rate or mechanism. In addition to granting these two points—which no careful worker can grant—it must be shown that the increase is independent of vascular changes within the skull or respiratory changes. An attempt to “standardize” the animal by estimating by experiment the influence of arterial, venous and respiratory changes leads into dangerous territory, because of the wide variations in different animals and in the

same animal from time to time, partly because of differences in the amount of fluid in the canal, partly because of the differences in reaction after the prolonged action of an anesthetic. This will be shown clearly in the experiments, later. We believe that most of what is observed in the experiments where the outflow method is used is the escape of preformed fluid. In some of the experiments it is squeezed out at an increased rate by a rise in the arterial or the venous pressure or both, and by an alteration in the respiratory rate. To this fluid is added, perhaps, some newly formed fluid arising under conditions of reduced pressure which exist in the canal because of the abnormal differences in pressure between the blood in the vessels and the fluid. This newly formed fluid must of necessity be of uncertain amount, the method gives no way of determining it or of distinguishing it from the rest of the fluid, and further it may be formed by a mechanism entirely abnormal, so that even if it were measured accurately, it would give no clue regarding the normal rate or the normal mechanism of the formation of the cerebrospinal fluid.

2. *Permanent fistulae.* Permanent fistulae were introduced first by Cavazanni (15) for the observation of the formation of the fluid under the condition of constant loss, and may be valuable for the study of the variations in the chemistry of the fluid if the infected animals are rejected. This method can give no data regarding the mechanism and rate of formation of the fluid over periods of long duration. The objections mentioned against the temporary fistula are valid here, and in addition the voluntary movements of the animal without an anesthetic would make the observation of slight variations of the formation of no significance whatever.

#### *Manometric methods*

The manometric method was introduced by Leyden (16) with excellent results. Both increased and decreased pressures are recorded, and any increased pressure of the fluid persisting for an appreciable time and independent of changes in the arterial and venous pressures in the skull, is interpreted as indirect evidence of new formation of the fluid. Dixon and Halliburton (12) used this method in their later work. The advantages to be pointed out are the following: This method avoids the error of incomplete emptying of the canal for the fluid is all retained within. It avoids the error due to the reduction of the pressure from partial drainage of the fluid from the canal. It

sets no maximum pressure within the canal, the pressure increases or decreases as in the intact animal. Only enough fluid need be removed to fill the manometer tube to the required height. For this reason it avoids the error due to new formation from abnormal differences between intravascular and intracranial pressures, when the pressure in the arteries and veins becomes too much higher than the fluid pressure. Thus the pressure of the cerebrospinal fluid varies within the normal limits of the intact animal and does not remain constant throughout the experiment as in the cases studied by the outflow method. If the process studied involves a fall in the pressure in the blood vessels, shown ordinarily by the recession of fluid in the tube, the manometer records this change as well as a rise in pressure, thus it is adapted to study both the pressor and the depressor phase.

However, when using the manometer method for studying the behavior of the fluid, certain precautions must be observed: A failure to do this has led to some surprising errors on the part of some observers. The needle connecting the canal with the manometer must be wide, otherwise there will be considerable delay in the rise of pressure in the cerebrospinal fluid manometer in response to changes in the venous and arterial pressures. This shows itself on the tracing by a rise in cerebrospinal fluid pressure which continues to become greater after the venous and arterial pressures begin to fall. When such a phenomenon occurs, naturally it need not be interpreted as meaning that the rise is not due to alteration in the arterial or venous pressure, because it is not synchronous with it, for it is due to those alterations. The significance which must be read into it is that there was an obstruction somewhere and the maximum ultimately recorded by the cerebrospinal fluid manometer is not the true maximum, but a pressure somewhat lower; lower by the amount of arterial or venous fall which occurred between the arterial and venous maximum and the cerebrospinal fluid maximum. This is of importance only in those cases where the pressure changes are very rapid in both directions as, for example, after adrenalin. Not all the trouble lies in the needle, a part may lie in the aqueduct of Sylvius and in other straits in the canal. Of course these latter errors cannot be avoided, and hence too much significance should not be placed upon the observation that changes in the fluid pressure are not absolutely synchronous with, or in the same direction as changes in arterial and venous pressures. We have observed arterial and venous pressures falling with the fluid pressure still rising, but such a rise was the result of the increased venous pressure, and stops short of



the height which would have been reached had the venous pressure become stationary when the high point was reached.

Great care must be exercised in the selection of the manometer tube in the case of the cerebrospinal fluid. If the bore is too small it offers too much resistance to the movements of the fluid and the influence of capillarity upon the level sought by the fluid is too great. If the bore is too large, the volume drained from the canal is too great, and the manometer shows a pressure which is not the pressure of the fluid in the intact animal, but the pressure of the fluid in the intact animal minus the amount of fluid withdrawn in filling the manometer tube. On the other hand if the manipulation involves a fall in pressure, and the bore of the manometer is large, the pressure recorded will not be the pressure of a normal animal under similar conditions, but will be the pressure of the normal animal with the canal overfilled by the volume of fluid equal to that which flowed in from the manometer. The importance of this point will be brought out and substantiated more fully. The use of a tambour to record manometric pressure is unsatisfactory. Most tambours leak, slowly it is true if proper precautions are observed, but rapidly enough, where delicate rubber is used, as in this case, to make standardization of little value. This is especially true if the pressure changes are of long duration either in positive or negative direction. The lever of the tambour traces the arc of a circle, not a vertical line, and hence the true time relations are in doubt. This is especially true if one tambour is set to write in one direction, the other in the other. Marked examples of this type of result are seen in graphs published in some of the latest work (12).

We finally come to the most fundamental objection to the use of the manometer to record variations in the cerebrospinal fluid pressure. These are two in number: the pressure conditions are such as to facilitate the absorption of the cerebrospinal fluid along the natural channels, hence the fall in the pressure of the cerebrospinal fluid observed in many cases may be due in part to vascular readjustment, in part to absorption of fluid along the natural channels, or either may be active independently of the other. In either case it is impossible to estimate the part played by each factor, if absorption actually enters into the problem, as must be the case if formation and absorption are as rapid as the clinical evidence given indicates. There is however good evidence that the rôle played by absorption in this readjustment is a minor one, for as will be shown later ligation of the jugular veins—along which it is agreed that most absorption takes place—does not

alter essentially the behavior of an animal in response to the injection of such drugs as pilocarpine. Further, the fall in the cerebrospinal pressure is synchronous with the fall in venous pressure and arterial pressure. The second plausible objection to the manometer method is the possibility that the cerebrospinal fluid is secreted under low pressures only, and hence might cease to be secreted at a pressure so low that it is impossible to indicate it by means of the manometer. This objection is not valid; it is evident from certain clinical cases that cerebrospinal fluid is secreted, or at least exists at high pressures; besides the manometer merely records the pressure and its physiological variations, and produces no new pressure of itself. Hence it cannot be blamed for raising the pressure above the limit at which fluid can be formed.

## II. EXPERIMENTAL METHODS EMPLOYED IN THE STUDY OF THE FLUID

It is our intention to describe in detail the methods used in our study of the cerebrospinal fluid.

*Anesthesia:* The effects of the alterations in the anesthesia are such as to make a constant degree necessary. We found constant slow intratracheal insufflation admirably adapted to this purpose. A constant degree of anesthesia is still more easily obtained if a dose of morphine is given preliminary to the anesthetic, but we avoided the use of the latter drug because of the tendency of an animal under its influence to show wide variations in the respiratory rhythm, with periods of apnea and dyspnea alternating, variations reflected upon the fluid by the production of undesirably wide fluctuations with each respiration, and alterations in the general blood pressure levels, with the periodic respiration. In our later work ether alone was given by inhalation, and proved perfectly satisfactory, when we employed a special valve prepared to give a uniform mixture of air and ether.

*Arterial pressure:* The arterial pressure was recorded by the mercury manometer from the left carotid or the femoral, in nearly all cases from the former. In some animals the pressure was taken in the circle of Willis according to the method of Hürthle (19). This pressure is not necessary in all cases because it usually follows faithfully the arterial pressure in the carotid, except where the cerebral arteries are ligated or in the cases of some of the drugs. The former requires no discussion, the latter will be taken up in detail later. For anything like accurate results in the circle of Willis, large dogs with

a wide internal carotid are necessary, and even in these the results are not always satisfactory. However, the method shows the changes in a general, even if not in an absolutely accurate way.

*Venous pressure:* It became evident early in this work that in these experiments the measurement of the arterial pressure alone is not enough to control alterations in the pressure of the cerebrospinal fluid due to mechanical factors. For this reason, especially for lack of the venous pressure in the head, some of the recent work is of little value in determining the mechanism of fluid formation. (Frazier and Peet (7), (14), and Weed and Cushing (8), (9)). Some of the conclusions are correct but that is true simply because venous pressures in the cases under observation follow the arterial pressures directly, and their experiments did not prove the facts in the case. The venous pressure must be taken from the head, for the pressure in the heart or in the superior vena cava has little or no relation to the pressure in the skull. (See table 1 and fig. 1).

In our experiments if we failed to establish venous pressure the animal was discarded as useless. We use a venous pressure method copied in part from Burton Opitz (17), in part from Hill (18), and modified to suit our own needs: a brass tube 1 cm. long and 4 mm. in diameter provided with a reservoir of about 40 cc. capacity, filled with a solution of sodium carbonate with a specific gravity of 1.088 is screwed into a drill hole made into the torcular herophili through the external occipital protuberance. The tube and reservoir are connected with a manometer filled with the same carbonate solution. Before opening the clamp and permitting the fluid in the bulb to communicate with that in the manometer, the pressure is raised in the latter to a point a few centimeters higher than that usually found in the sinus, so as to force a small amount of the solution into the sinus, and thus avoid the clotting in the system from the accumulation of blood in the short tube between the sinus and the reservoir. If this precaution is observed venous pressure can be studied for hours without enough clotting in the apparatus to make the error appreciable. A *post mortem* must be made in every case to rule out clotting, for with carbonate solutions the whole sinus system may be filled with a fine membranous clot which markedly interferes with the venous outflow, and it is common to find in many experiments venous and fluid pressures rising slowly but steadily throughout the period of observation. The mechanism recording on the drum includes a three or four unit signal magnet. An observer watches the level of the sodium carbonate in the manometer

and records the variations in pressure by closing the key in one magnet circuit, once if the level rises past a centimeter mark on the scale, twice if it falls below the mark. In our earlier experiments the manom-

TABLE 1

*This table shows the relation between arterial pressure, venous pressure in the torcula, and venous pressure in the stump of the axillary vein, where the latter joins the jugulars*

	ARTERIAL			GENERAL VENOUS			TORCULAR VENOUS		
	B.	D.	A.	B.	D.	A.	B.	D.	A.
Pilocarpine.....	114	{ 86 140	104	85	155	100	90	130	110
	122	{ 100 160	104	15	30	15	105	269	105
	78	94	70	-25	-30	-25	40	85	40
	116	{ 92 146	110	-5	70	0	10	19	10
	112	{ 70 170	90	5	84	5	225	315	220
	140	{ 112 160	130	1	1	1	5	240	100
	130	{ 30 140	110	1	1	1	235	300	240
Nicotine.....	80	170	32	3	3	3	30	38	30
	92	150	98	0	-1	1	20	26	22
Adrenalin.....	100	210	92	0	0	0	12	19	12
	64	240	58	-35	-35	-35	25	285	95
	60	246	78	-1	-8	-1	5	210	10
	134	278	118	1	-8	1	11	39	12
	80	168	80	2	5	2	7	18	14
	80	186	74	3	6	2	12	27	12

B = Before the action; D = During the action; A = After the action of the drug. Where two figures are given, these figures represent the minimum and maximum.

eter was connected with a tambour covered with the thinnest rubber obtainable, in addition to the record described. In most of our later experiments the tambour was discarded because errors from leak-

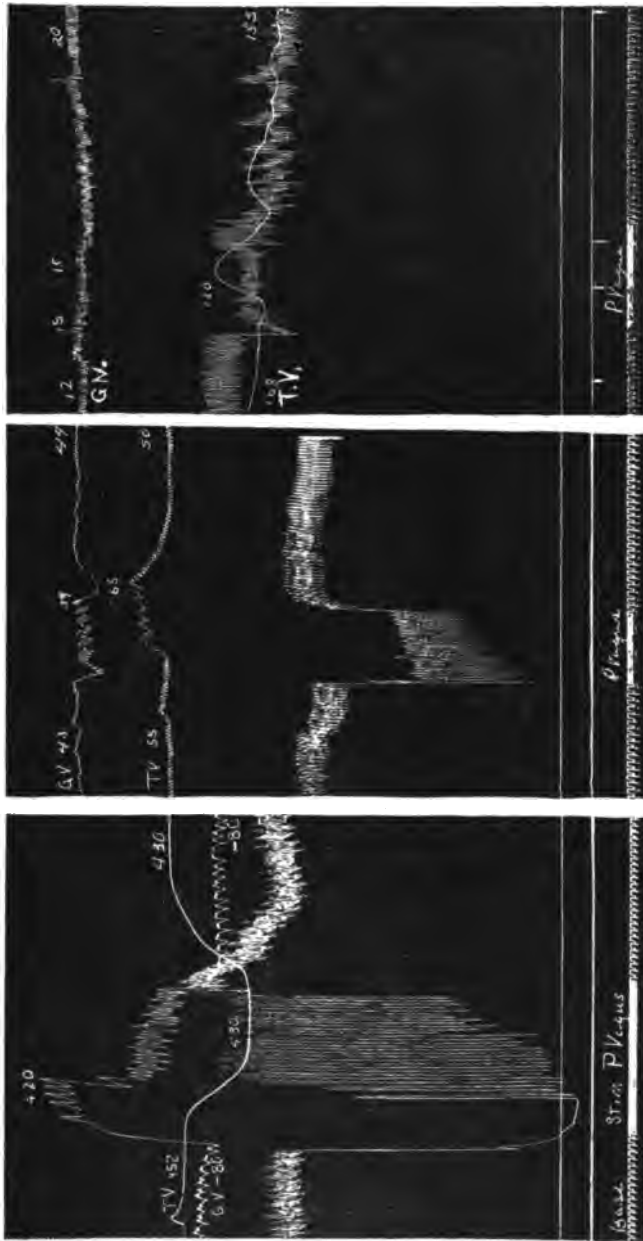


Fig. 1. These tracings show the different reactions of general and cerebral venous pressures in different animals in response to the stimulation of the peripheral vagus. A comparison of torcular, *T. V.*, and general, *G. V.*, pressures shows marked differences in behavior and emphasizes the importance of the measurement of venous pressure in the skull, not in the great veins of the body, when conclusions are to be drawn regarding the cause of pressure changes in the head.

age made the record untrustworthy, time relations were altered, and in addition we found it advantageous to use a small manometer tube, the variations of which were too small to give a suitable tracing. In no case did we depend upon the tambour to determine the actual variations in pressure, since this can be read directly from the magnet record. In addition from time to time—e.g., when we knew by experience that the maximum or minimum point had been reached, or at regular intervals—simultaneous venous and fluid pressure readings were made, and recorded directly on the tracings after the paper was removed from the drum. General venous pressure was recorded in some cases from the axillary vein, where it joins the jugulars. We avoided the right auricle because of difficulties from clotting, but mainly because we did not want to interfere with venous outflow from the brain by cutting off one of the jugulars, or by occluding even in part the superior vena cava.

*Cerebrospinal fluid pressure:* In our determinations of the behavior of the cerebrospinal fluid under various experimental conditions we employed two methods: The first was a simple one we devised, only to find later that it was essentially the method used by Spina (10). The animal is placed on its side on a level table. The trocar is inserted into the *cisterna magna*, and after the obturator is removed the trocar is connected at once by means of a T-tube to a heavy piece of glass tubing 1 m. long with a uniform internal bore of 1 mm. This glass tube is fastened on a meter stick with centimeter markings, which furnishes the scale. The tube is then adjusted on stands so that the lower end attached to the needle is at exactly the level of the point of puncture of the occipito-atlantal ligament; the upper free end is adjusted to the level of the upper border of the cerebral hemispheres. Thus there is provided a water gauge showing pressure variations of the liquid in the dural canal. The fluid escaping while the connection is being made is collected and replaced into the system through the T-tube. This step is important for during the early part of our work this point was not appreciated and in many cases the early tracings were marked "animal refractory to drugs." Later it was found that a refractory animal became a susceptible one if the canal was filled with fluid (see table 15). As in the case of venous pressure, an observer watches the column of fluid oscillating in the tube and records the variations in the same way by closing a key in the proper magnet circuit. While this method requires the constant attention of one person, it is accurate because any blocking of the passage to the canal is noted at once by the disappearance of the oscillations. This method does not reduce the pressure by drain-

ing away fluid, complete drainage of the preformed fluid is not required nor attempted. There is no resistance to backflow of fluid produced by an artificial resistance as in the Weed-Cushing method. No maximal limit is imposed during the experiment; instead the pressures are maintained absolutely normal, the fluid is free to flow outward or inward depending upon whether the pressure within the skull is increasing or decreasing.

The only real objections to be advanced are the ones already pointed out against the manometer method: The fluid is under normal pressure, thus there may be absorption along natural channels; the pressures may be such as to inhibit the normal mechanism. However, it will be shown later that neither of the objections is valid.

While it would seem reasonable that the method described for recording variations in the cerebrospinal fluid would be adapted to the purpose of this research, it was necessary to determine the accuracy. To do this, experiments in which simple mechanical factors are known to be the only ones involved were undertaken. The most direct test imaginable is the direct injection of fluid into the dural canal. With the tube in place and connected in the manner described, numerous experiments were carried out. There were considerable variations between different animals but results in the same animal were uniform. Thus in one experiment the introduction of 4 cc. of warm 0.9 per cent sodium chloride solution changed the position of the fluid from 20 to 80. The withdrawal of 4 cc. brought the level of fluid again to 20. Repetition of this experiment numerous times showed that the error was less than 1 cm. on the scale, or in volume less than 0.031 cc. Of the 4 cc. increase in fluid 1.86 cc. appeared in the tube. In this case the needle through which the fluid was injected was 1 cm. from the trocar connected with the gauge. Injection of fluid in other parts of the canal produced less accurate results unless the injection was made into the third ventricle through the corpus callosum. In the latter case the results were almost as accurate as in the case of injection into the *cisterna magna*. Tests made with the manometer were equally satisfactory.

The second method employed was the manometer method already discussed. Our manometer had a bore of 1 mm., thus the question of draining the cavity if the fluid pressure is rising, or of overfilling it if the pressure is falling cannot enter to any degree. We at first used it as a check on the tube method described, but later we used it in most of the experiments because of the advantage that this method enables us to draw direct comparisons between fluid and venous pressures.

The medium used in the manometer was a sodium chloride solution with a specific gravity of 1.088 so as to make it directly comparable to the venous pressure. Sodium carbonate solution could not be used because of its toxicity if introduced intra-durally. Variations in the fluid pressure in this method were recorded in the same way as described for venous pressure.

Before entering upon the details of the experimental work, we wish once more to emphasize the importance of recording arterial, venous and cerebrospinal fluid pressures continuously and simultaneously in every experiment. In some experiments the pressure in the circle of Willis is also essential. The continuous and simultaneous record is of the greatest importance, because of the unique conditions existing within the skull where is found the brain, incompressible to ordinary pressure, surrounded by the undilatable skull, and separated from it by the membranes, blood vessels and the cerebrospinal fluid. Any change of either venous or arterial pressure must be followed either by variations in pressure in or by variations in the amount of the cerebrospinal fluid. Hence in order to control properly the mechanical factors, both arterial and venous pressures must be measured, and the venous pressure because of its variability and because of its great direct influence upon the pressure is relatively, so far as the cerebrospinal fluid pressure is concerned, much more important than the arterial. The cerebrospinal fluid pressure is the sum of the influence of the arterial and venous pressures, as we will be able to prove later in the paper, so if both pressures rise the fluid will flow out in the tube, or will rise in the manometer; if both fall the fluid recedes in the tube or falls in the manometer. If one rises and the other falls—a situation which does occur under experimental conditions, actual measurement, not theory, will determine whether the cerebrospinal fluid pressure will rise or fall. From this statement of the situation the importance of these measurements becomes clear. We would not have had to dwell upon this point if the recent workers had read more carefully the excellent monograph by Hill on "The cerebral circulation." Because of additional data we ascribed more importance than Hill does to the direct influence of arterial pressure upon the cerebrospinal fluid pressure.



## III. THE MECHANICAL FACTORS INFLUENCING THE CEREBROSPINAL FLUID

*Introduction*

A careful experimental study of the mechanical factors is imperative to establish the necessary controls for the work on the drugs and the tissue extracts, as well as to obtain a definite, concrete idea of the factors of error in the method employed. Provided by simple experiments with the controls and the factor of error for which we must allow, we will then be in a position to interpret the mechanism of action in the more complicated experiments. If a drug or a tissue extract gives a result which can be explained rationally on a mechanical basis, we are not justified in assuming that any other factor is at work. Only after the effect of a drug on the fluid cannot be explained on a mechanical basis, and not until then, is there any reason for ascribing to the substance a stimulating or depressing effect upon the mechanism generating the fluid.

In this section we intend to study the conditions of pressure that are found to exist in the normal animal, the various changes undergone by these pressures during long experiments under ether, and the mechanical forces which may influence the behavior of the cerebrospinal fluid. Of the latter the following are the more important: cerebral venous pressure, arterial pressure, respiration, the volume and pressure of the fluid in the skull, and the action of the vagus. Some of these clearly will have a direct, others only an indirect effect upon the fluid and its movements. No experiments by pressure methods upon the action of drugs and of tissue extracts in augmenting or inhibiting the formation of the cerebrospinal fluid can rest upon a satisfactory basis unless the natural relations of arterial, venous and fluid pressure are accurately known. It is also necessary to know what effect increasing or decreasing each of these pressures one or more at a time has on the others. Unless these facts are known it will be impossible to differentiate cause from effect. But when these facts are known, then it will be possible at least in part, in other and more complicated experiments to determine to what degree the purely mechanical changes of the arterial and venous pressure will account for the changes in the fluid pressure. In a case where the arterial pressure remains constant and both venous and fluid pressures rise, it is not possible without experiment to tell whether the pressure in the former rises because of the rise in the latter or *vice versa*, or whether they rise simultaneously but independently. If, however, experiment shows that raising the venous pressure raises the fluid pressure, but

raising the fluid pressure moderately does not raise the venous pressure, then we are justified in concluding that venous pressure influences the fluid pressure, but the fluid pressure does not influence the venous pressure.

At the beginning of the work it was hoped that in any one dog a certain change in the pressure in the arterial or the venous blood would always produce some certain change in the fluid pressure. Then, if the fluid pressure rose or fell more than was to be expected from the change in the vascular condition, it would be safe to assume increased formation or increased absorption. Unfortunately, these hopes were not realized. The amount of the influence varies widely in the same animal from time to time as will be shown conclusively later in the paper. For this reason the attempts to "standardize" a dog were abandoned after a few trials. Attempts to do this in the past have led to erroneous conclusions. If the reader will keep this in mind he will not be surprised in the apparent discrepancies in some of the experiments to be listed in our tables later.

#### *1. The normal levels of venous, arterial and cerebrospinal fluid pressures*

Having accepted the view that the best method of determining variations in the amount of fluid is the indirect one, by studying the pressure of the fluid in the canal, it is clear that a large number of measurements of normal pressures must be undertaken in order to obtain some idea of the limits of variation. This was done, and with the fluid pressure were made simultaneous measurements of arterial and venous pressures.

*Literature:* That the fluid is under positive pressure is a fact long well known. Leyden (16), probably the first to make measurements at all accurately, reports 70, 480, 18, 15 and 90 mm. of water in the 5 dogs measured. When measured with the mercury manometer the pressure was always higher, comparatively, a difference which he ascribes correctly to the loss of fluid into the manometer tube. Falkenheim and Naunyn (19) report the fluid pressure as exceedingly variable: the pressure usually varied between 30 and 38 mm. of water, but they found one dog with 140 mm. of water pressure, another with 75 mm. and another with 15 mm. They believe the pressure to be independent both of the size of the animal and the arterial pressure. Hill (18, p. 73) says: "In normal conditions the pressures generally are 100 to 130 mm. of water." Dixon and Halliburton cite figures about as follows: 190 mm.

10 per cent magnesium sulphate, 45 mm. normal saline; others with 150, 150, 150, 180, 80 and 60 mm. were observed. The exact amount of this pressure, therefore, and its relationship to the venous and arterial pressures is still a matter of some doubt, because there are wide variations in the figures given by the various workers, and in different experiments by the same worker. As can be seen from our work, the reason for such variations is the fact that the pressures normally vary within wide limits in different animals. The best that can be done is to give the readings from a large number of animals and the average from these experiments. See table 2, where we record the first measurements of arterial, venous, and fluid pressure in thirty-nine animals measured according to the method described above. As can be seen from the table the average arterial pressure was 128.79 mm. of mercury, the average venous pressure was 124.97 mm. of sodium carbonate (1.088), and the average cerebrospinal fluid pressure was 112.25 mm. of sodium chloride (1.088). The highest pressure observed in the fluid was 242 in 12, and the lowest was 43 in 219; the highest venous pressure was 425 in 155, the lowest was 44 in 219. It is obvious that the venous and cerebrospinal fluid pressures vary within wide limits, in different animals, even if the same methods are used in making the measurements. This accounts for the wide difference in the figures cited in the past, and shows that the pressure limits are by no means fixed. Twenty-six of the thirty-four dogs measured were below the average given.

We are fully aware of the fact that the figures in our table differ in one essential detail from those given in the past for in nineteen of our thirty-nine dogs the fluid pressure is higher than venous pressure. Hill says: "The intracranial (c.s.f.) pressure has been found in all physiological conditions to be the same as the venous pressure," and adds: "By no physiological means can intracranial pressure be maintained higher than cerebral venous pressure" (p. 68). The data afforded by our experiments shows that Hill's conclusions regarding the equality of the intracranial and venous pressures are not substantiated by our experiments. Either may be higher than the other, but under normal conditions the differences in pressure are small, and the variations in pressure in experiments free from error usually occur in the same direction, although not always exactly to the same degree. Therefore, since our findings are based on experiment, Hill's conclusions on theoretical grounds from experiments on absorption, we are forced to the conclusion that his statement is in error. Frazier and Peet (14) hold venous and fluid pressure to be identical.

TABLE 2

*The normal levels of arterial, venous and fluid pressures. This table shows the first readings on 59 dogs and represents, therefore, the normal levels of the arterial, venous and fluid pressures.*

TRACING NUMBER	ARTERIAL PRESSURE	VENOUS PRESSURE	C. S. FLUID PRESSURE
	mm. of Hg.	mm. sod. carb.	mm. sod. chlor.
3	153	136	143
5	134	77	109
6	123	75	82
7	154	322	187
8	138	85	96
9	130	125	158
10	116	87	84
12	146	272	242*
14	112	285	104
15	114	81	95
17	112	102	77
18	132	105	125
20	168	56	44
150	130	262	211
151	176*	118	214
155	153	425*	169
171	142	84	56
174	108	205	98
184	140	116	96
192	124	141	123
197	148	150	99
214	110	72	83
216	130	55	113
219	104	44†	43†
229	108	75	72
230	130	208	90
231	100	63	68
243	120	78	109
258	154	98	140
267	124	127	97
274	92†	46	61
277	88	67	56
279a	160	108	160
279b	124	98	97
280	100	82	100
281	122	87	95
282	170	90	106
283	90	95	110
284	144	72	166
Average.....	128.70	124.97	112.25

\* Highest pressure.

† Lowest pressure.

In consequence of what we have shown the last part of the following statement made by Dixon and Halliburton (12) is not correct: "One law always holds, however, under ordinary conditions, the arterial pressure is higher than the venous pressure, and the venous pressure than the cerebrospinal fluid pressure." This law does hold for some of the cases; but numerous variations are seen in which the fluid pressure is higher than the venous pressure in the torcula. We have seen this so often in our experiments that we are confident that it cannot be an error due to manipulation. As may be seen in table 2, in which the data from thirty-nine dogs is listed, when the observations were begun the pressure was higher in the venous blood than in the fluid in twenty cases, the pressure was higher in the fluid than in the venous blood in nineteen cases.

We do not believe that a fluid pressure much higher than the venous pressure is normal, but it does happen frequently that a pressure in the fluid higher by a few millimeters than in the venous pressure is found. Observations with the fluid pressure much higher than the venous pressure were made; in most but not all of these cases epidural or subdural hemorrhages were found. Why an epidural hemorrhage should cause a higher fluid pressure will become evident later. Such a hemorrhage is usually but not always of arterial origin. For that reason we found it best always to discontinue an experiment when a great increase of the fluid pressure over the venous pressure was observed as well as in cases where the venous and fluid pressures were always equal. The latter observation always means a communication between the subarachnoid space and the sinus. On the other hand cases were found in which such a communication existed, but in which the pressures were not the same at any time in the experiment.

Since our results are not in agreement with those of our predecessors we must point out errors in their experiments which we were able to avoid. We believe that we can do this satisfactorily. Neither of the authors quoted above mention at any point in their papers the size of the manometer tubing used in their measurements. Leyden, practically the first investigator to measure the fluid pressure carefully, pointed out the importance of the loss of fluid into the tube in the determination of fluid pressure. He ascribes the lower readings obtained from the water manometer as compared with readings obtained with the mercury manometer in the same animal to this cause. His objections and views in the matter are correctly taken. Of course if care is taken to adjust the level of the fluid in the tube to such a height as to balance the pressure

in the canal, before the latter is connected with the manometer, so little or no fluid can escape from one to the other, then the first reading will be approximately correct. However, even if this precaution is observed, error can not be avoided later in the experiment if a large bore manometer is used, an error becoming progressively greater the farther the pressure in the canal varies from the normal. Therefore we conclude: if a large bore manometer is used a correct estimate of the pressure of the fluid can be obtained only if the adjustment of the fluid to the pressure in the canal is nearly perfect at the time the needle is pushed into the canal.

If this situation holds for the normal pressure, it is clear that with rising or falling pressures, this factor becomes more important the farther the pressure deviates from the normal. A consideration of the situation shows: Under ordinary conditions where there is an abundance of fluid to supply the apparatus when the pressure is rising, and an abundance of room to accommodate the fluid when the pressure is falling as is the case in the vascular system, the bore of the manometer used in making pressure measurements is of no importance. This holds true in taking venous pressures, for in the cerebral venous system there is an abundance of blood available from the general circulation to supply the manometer tube, when the pressure is rising, and plenty of space to accommodate the excess of fluid when the venous pressure is falling. On the contrary, when dealing with a situation where the amount of fluid as well as the space to accommodate fluid is limited, as is the case with the cerebrospinal fluid and the dural canal, the bore of the manometer is of importance. With the escape of the fluid from the canal into the tubing while the pressure is rising, the observer will measure not the normal pressure in the canal with the normal amount of fluid, but the pressure in the canal less the pressure which would have been produced by the amount of fluid lost into the system of tubes. When the pressures fall below the normal, he measures not the pressure in the canal under a similar condition in the intact animal, but the pressure in the canal increased by the amount due to the excess of fluid entering from the manometer tubes. For this reason a large bore manometer will measure the true pressure of the venous blood in the sinuses, but will not measure the true pressure in the fluid under normal conditions in the dural canal, if the pressure varies in any degree either above or below the initial level at which the observations were begun. To test out the validity of this point we performed the experiments shown in table 3.

This table shows clearly that the bore of the manometer influences markedly the results obtained in the measurements of the pressure of the fluid. This does not hold true to the same degree for venous pressure measurements. The fact that the tracings shown by Hill and by Dixon and Halliburton, show wide excursions of the tambour levers, is evidence that their manometers were fairly large, and for that reason their records of the fluid pressure where the pressure is rising are too low, where the pressure is falling too high. Because in the former case a part of the fluid flows out to fill the tube partially emptying the canal and reducing the final pressure below what it would have been if the

TABLE 3

*Influence of the bore of the manometer on the readings of venous and cerebrospinal fluid pressures. (All three manometers were connected before beginning any of the readings with the fluid level at zero. The measurements were made by opening the clamp to 1, and taking the reading; the fluid was then forced back into the canal so the fluid stood at zero, and the clamp was closed; 2 and 3 were then read in the order named. Manometer 1 had a bore of 1 mm.; 2, 5 mm.; 3, 8 mm.)*

	GEN- ERAL ARTE- RIAL	TORCULAR VENOUS			C. S. FLUID		
		Manometer number					
		1	2	3	1	2	3
Normal.....	102	127	115	115	97	89	89
During occlusion of the jugulars.....	100	237	236	230	157	96	90
Normal after release of jugulars.....	98	126	110	110	94	90	84
Normal.....	160	445			162	54	27
Normal.....	160	401			95	44	15
Normal.....	84	116	119	116	60	11	11
Normal.....	160	90	80	79	36	37	35
Occlusion of jugulars.....	160	204	173	172	110	81	57

normal amount of fluid had been in the canal; in the latter the fluid flows from the tube into the canal overfilling it, and raising the pressure above what it would have been if the normal amount of fluid had been in the canal.

On the other hand the conclusions of Wegfarth (20) from the work of his predecessors as expressed in his statement (p. 164): "It seems probable that in both situations (the eye and the brain) the fluid pressures are constantly being reduced to the level of the venous pressure in the great sinuses, and that, because of the resistance of the complex fluid pathways the fluid is constantly at a higher pressure than in the sinus,"

are not warranted by our findings nor the findings of our predecessors. Since it is certain that venous pressure usually is—some say always—higher than cerebrospinal fluid pressure, it is self-evident that the establishment of a free communication between the venous sinuses and the subarachnoid space with the idea of furnishing a pathway of escape for the fluid in the case of hydrocephalus as suggested first by Gaertner (21), and modified later by McClure (22), Haynes (23) and Wegefarth, is not an operation to be undertaken lightly in man because of the obvious dangers of subarachnoid clot formation and its sequellae as soon as the pressure in the skull falls to the normal.

Our final conclusion regarding the levels of arterial, venous and fluid pressures are: Venous and fluid pressures are always less than arterial pressure. Venous and fluid pressures are almost but not exactly equal, but no fixed law can be given in regard to which is under the greater pressure. The differences when great are in favor of the venous pressure, and we find a good many normal animals in which the venous pressure is considerably higher than the fluid pressure (100 mm.). In our series of thirty-nine experiments here listed, seven animals with such pressure relations were found. The use of a manometer with a wide bore led to the failure of earlier workers to discover that venous and fluid pressures are not identical, and that it is physiologically possible at least in the dog for the fluid pressure to be higher than venous pressure.

## *2. The normal variations in the arterial, venous and cerebrospinal fluid pressures in the anesthetized animal*

Having established the normal levels of arterial, venous and fluid pressures it is necessary to study the variations undergone by these pressures during a long experiment to see how the pressures of these fluids are modified by continued anesthesia. Further, it is essential to see whether the initial levels of these pressures are representative values, for it is conceivable that they are subject to such wide independent variations that the initial levels are not significant in the least degree.

*Literature:* Hill believes the intracranial (p. 71) "is the same as the cerebral venous pressure, and varies in the same direction absolutely as general venous pressure, and proportionately as general arterial pressure" (p. 72). The "cerebrospinal fluid pressure does not always absolutely correspond, since the brain . . . on expansion expresses the cerebrospinal fluid from the cranium and comes in contact with the cranial wall."



Dixon and Halliburton (12) in their discussion of the normal fluid pressure cite in detail an experiment which gives the impression that very great differences exist between the venous and the fluid pressures, normally, and that wide variations in the fluid and venous pressures are common. At the beginning of, and during an experiment lasting 75 minutes, these pressures showed wide variations. Summarized the experiment is as follows:

	BEGINNING	AT 35 MINUTES	AT 65 MINUTES	AT 75 MINUTES
	mm.	mm.	mm.	mm.
Venous.....	350	415	330	255
Fluid.....	95	65	50	90
Difference.....	255	350	280	165

It should be noted that between the first and second observations the venous pressure rose and the fluid pressure fell; between the second and third, they both fell; between the third and fourth the venous pressure fell and the fluid pressure rose. Without citing definite figures the authors say that this experiment is typical of all other experiments. Thus venous and fluid pressures varied twice in opposite directions and once in the same direction in three observations.

TABLE 4

*This table shows the variations in pressure in an animal under anesthesia for three hours. Observations were made at 15 minute intervals*

	1	2	3	4	5	6	7	8	9	10	11	12	13
General arterial.....	90	100	106	104	106	112	112	112	116	114	120	128	128
Circle of Willis.....	72	78	84	82	84	86	88	92	98	98	92	110	106
Torcular.....	95	92	78	87	105	130	145	112	128	115	182*	99	146
Fluid pressure.....	110	99	81	88	89	111	137	109	128	116	192	104	145
Venous minus fluid.....	-15	-7	-3	-1	16	19	8	3	0	-1	-10	-5	1

\* Cause of marked venous and fluid rise at this point not clear.

We have made observations on a large number of animals. In order to bring out the characteristics of our observations we will present the variations in detail in one animal. (See table 4.)

As can be seen from table 4 there is a gradual slow rise in all the pressures from the beginning to the end of the experiment. The arterial rise is gradual and steady; the fluid and venous pressure underwent

numerous fluctuations, but after the first 30 minutes the tendency toward a rise became well marked. The remarkable parallelism between venous and fluid pressures shows clearly in figure 2, which was obtained by plotting the readings on coördinate paper. The differences between venous and fluid pressures are much smaller than those reported by Dixon and Halliburton, the variations in venous and fluid pressure are more nearly proportional than reported by them and unlike in their experiment, they vary in the same direction in every instance. There are in this experiment no exceptions to this rule.

To confirm the data from this single experiment we give table 5.

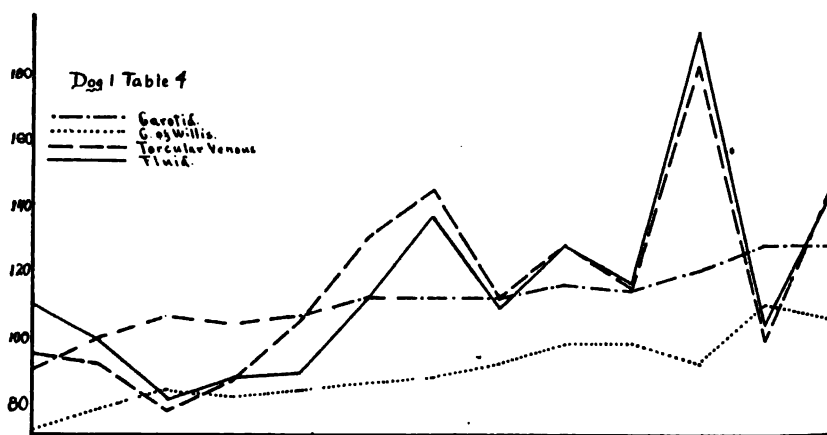


Fig. 2. This figure shows the alterations in pressure in the arterial, venous and fluid system during a long period of observation. No manipulations were performed throughout the whole period.

This table shows readings taken at 15 minute intervals from five dogs selected at random from our series of long time experiments. These variations should then be illustrative of the facts regarding the variations in the pressures in animals, in which mild changes were produced, and the animals were kept long under observation, and these constitute the controls for the whole work. As can be seen the variations in these five animals are practically similar with the variations in the single animal cited in table 4.

It is evident from the data in our tables that we cannot agree that the data in the experiment cited by Dixon and Halliburton is typical of a large number of experiments. In our experience the average fluid and venous pressures are much more nearly equal than in the experience

TABLE 5

*This table shows the variations of the pressures in animals under experimental conditions. Readings were made at 15 minute intervals. Note the variations in the relations of venous and cerebrospinal fluid pressures, either may be higher than the other, but closely follow each other. Arterial pressure in millimeters of Hg., venous pressure in millimeters of sodi. carb. (1.088); cerebrospinal fluid in millimeters of NaCl (1.088). Tissue extracts were administered between readings*

DOG NUMBER		OBSERVATION									
		1	2	3	4	5	6	7	8	9	10
1	General arterial.....	154	126	160	136	156	160	146	142	82	
	Circle of Willis.....	116	118	128	130	142	140	134	132	70	
	Torcular venous.....	87	101	130	67	65	66	61	59	44	
	Fluid pressure.....	84	95	115	48	42	51	56	64	49	
	Venous minus fluid pressure.....	3	6	15	21	13	15	5	-5	-5	
2	General arterial.....	130	118	116	120	122	116	100	82	88	80
	Circle of Willis.....	119	105	100	99	107	104	90	73	72	72
	Torcular venous.....	125	97	105	113	109	99	112	82	93	92
	Fluid pressure.....	158	131	139	140	132	116	137	111	116	115
	Venous minus fluid pressure.....	-33	-34	-34	-27	-23	-17	-25	-29	-23	-23
3	General arterial.....	146	128	146	148	152	156	152	126	114	
	Circle of Willis.....	90	80	84	118	118	116	102	76	66	
	Torcular venous.....	272	246	276	301	307	319	296	251	234	
	Fluid pressure.....	249	228	257	270	282	292	287	245	228	
	Venous minus fluid pressure.....	23	18	19	31	25	27	9	6	6	
4	General arterial.....	124	120	124	132	130	120	122	130	124	122
	Circle of Willis.....	96	96	94	100	104	98	96	104	100	92
	Torcular venous.....	75	75	78	83	65	53	50	54	55	71
	Fluid pressure.....	82	77	79	85	69	53	47	48	52	65
	Venous minus fluid pressure.....	-7	2	-1	-2	-4	0	3	6	3	6
5	General arterial.....	138	148	140	150	178	152	162	156	154	137
	Torcular venous.....	85	124	90	95	114	59	88	111	118	105
	Fluid pressure.....	96	131	105	88	132	48	79	115	117	106
	Venous minus fluid pressure.....	-9	-7	-15	7	-18	11	9	-4	1	-1
Average from Dogs 1 to 5											
Average..	General arterial.....	138	124	137	137	147	141	136	127	112	113
	Circle of Willis.....	105	100	101	112	118	114	105	96	77	82
	Torcular venous.....	129	129	136	132	132	119	121	111	109	89
	Fluid pressure.....	134	132	139	126	115	112	121	117	112	96
	Venous minus fluid pressure.....	-5	-3	-3	6	17	7	0	-6	-3	-6

of Dixon and Halliburton. We have a much closer parallelism between the changes in the two pressures than is shown by their experiments. In our experiments, as can be seen by reference to the tables, the variations in the normal animal are almost always in the same direction and are to some degree proportional. The variations in all the pressures are greater in the experiment of Dixon and Halliburton than those seen in the animal under simple anesthesia. If these figures shown by these authors were typical, if the pressures were subject to variations as wide as those recorded, if the pressures lacked the parallelism of change to the degree their experiment shows, then it would be useless to go farther, for it would be impossible to draw any conclusions from experiments on this subject for the normal variations would be so wide as to render conclusions from experiments of no value. Our results show that the pressures are much less variable, and that the pressures vary much more nearly in the same directions and to the same degree. To be specific: In the 58 simultaneous changes shown in table 5 above, the pressure of venous blood and fluid varied in the same direction 50 times, in opposite directions 6 times, and in 2 instances one varied and the other remained stationary. In the same 58 changes arterial and venous pressure varied in the same direction 37 times, in opposite directions 16 times, and in 5 cases one remained unchanged while the other changed. In the same 58 readings arterial and fluid pressure varied in the same direction 39 times, in opposite directions 15 times, and in 4 cases one remained stationary while the other changed. It is thus self-evident that fluid pressure follows venous pressure more closely than it follows arterial, and also that venous and fluid pressure vary in the same direction and these variations are to some degree proportional.

Our variations in the pressure level of fluid is considerably wider in the individual animal than those reported by Weed and McKibben (36) in their recent article on fluid pressures. However, these variations add to rather than detract from the proof that the fluid follows venous rather than arterial pressure variations. Further, our composite curve of five animals, if plotted from table 5, would compare very closely to the curve they show as the composite of seven animals.

### *3. The influence on the fluid pressure of raising and lowering the venous pressure*

As has already been shown, the pressure of fluid in the canal is always positive under the conditions we have studied. The sources of this positive pressure must now be investigated, and if more than one force

contributes, then the relative importance of each must be determined by experimental, not theoretical, means. Unfortunately it is not always possible to secure a change in one factor at a time, leaving all the others constant, for any manipulation changing one, modifies to some degree one or more of the others. Hence the experimental proofs are not purely objective, and are weakened by the errors common to the interpretation of complicated experiments. In considering the origin of the positive fluid pressure, the following sources must be emphasized: *a*, arterial blood; *b*, venous blood—either or both of these may transmit pressure to the fluid through the walls of the vessels; *c*, alterations in the volume of the brain, from gain or loss of blood or other fluid by that organ; *d*, secretory pressure from the activity of the cells elaborating the fluid, thus forcing the accumulation of the fluid into a confined space, the canal. Any or all of these forces may be active in modifying the pressure under which the fluid is found. The influence of the factors enumerated is modified by the amount of fluid in the canal, and hence by the rate at which the fluid is formed and removed. The inter-relations of the arterial, venous and fluid pressures were studied by noting the variations in these pressures in an animal under ether for a long time without any other manipulations than those essential to the connection of the necessary apparatus, and by observing what effect modifying one at a time or two at a time had upon those remaining.

*Literature:* So far as we are able to cover the literature, Falkenheim and Naunyn (19) were the first to study the influence of raising the venous pressure on the pressure of the cerebrospinal fluid. They produced this result by blowing up a rubber balloon in the right ventricle. They noted in two of the cases a rise in fluid pressure; in the third, a fall. In the first two cases reported the arterial pressures were low, 105 to 120, in the third the arterial pressure was high, 210, and they concluded that the rises were due to venous stasis without much reduction in cardiac output, while the fall was due to a fall in arterial pressure due to the marked reduction of cardiac output because of the pressure of the balloon. These explanations are probably correct, although so far as the tabular report of the experiment is concerned neither venous pressure nor arterial pressure is recorded.

Dixon and Halliburton (11) in their first article dealing with the outflow of fluid under the influence of depressor substances in general and choroid extract in particular, eliminate the factor of the venous pressure by argument, not by experiment. They say (p. 240) in regard to depressor substances: "It might be argued that the fall of blood pres-

sure in the arteries will be accompanied by a rise in the veins. . . . This argument may be met in the following ways: (1) increase in the venous volume will be counteracted and probably counterbalanced by decreased arterial volume, so the change of brain volume will be negligible; (2) the fall of arterial pressure is not always seen; and (3) other substances which cause a depressor effect do not increase the cerebrospinal flow." That this argument will not hold will be shown in the experimental part of the paper.

In their second paper (12) these authors had come to recognize the important influence of venous pressure on the fluid pressure. Thus they noted (p. 141) that a rise in venous pressure produced a rise in fluid pressure. "It will be noted that in each instance the rise in C. S. pressure is only about one-quarter that which occurs in the torcular." They say further (p. 142): "If arterial and venous pressures are lowered, as for example by bleeding, the drop in C. S. pressure is relatively small." They state further from experiment that the alterations in fluid pressure and venous pressure are not synchronous nor proportional, and hence independent of each other; and state in their conclusion: "The C. S. pressure is influenced passively to a small extent by changes in arterial and venous pressures, but such alterations are insignificant compared with the independent changes in pressure which occur as the result of secretory activity."

In their third article these authors study the effect of increased intracranial pressures upon the circulation and respiration. In addition to studying the effect on the various centers of the medulla they attempt to draw conclusions regarding the rate of blood flow through the brain by study of the rate of flow from the "cerebral end of the cut left internal jugular vein."

Frazier and Peet (7) state: "The pressure of the cerebrospinal fluid is under normal conditions identical with venous pressure, conditions affecting venous pressure . . . should disturb the pressure of the cerebrospinal fluid." From experiments briefly cited they conclude: "The pressure of the cerebrospinal fluid drops to zero after occlusion of the carotid circulation only to recover itself in a very few minutes, while occlusion of the venous circulation is followed by a rise in pressure more or less constant. . . . The more or less constant rise in pressure when the venous circulation is interrupted confirms the hypothesis that the absorption of cerebrospinal fluid takes place chiefly through the venous channel."

In a second paper (14) they state: "The administration of any depressor substance such as extract of spleen, ether, amyl-nitrate (nitrite?) or magnesium sulphate caused a marked drop in arterial pressure followed by a slow rise to normal. Practically coincident with this drop in arterial blood pressure was a sudden rise in the cerebral sinus pressure. This usually occurred immediately after the sudden drop, and not with it. The sinus pressure continued to rise as long as the arterial blood pressure remained at its lower level. As the femoral pressure gradually returned to normal the sinus pressure slowly dropped." They conclude: "The sudden increase in the rate of outflow following the injection of organ extracts is the result of sinus distension which forces out fluid already present in the ventricles and cisterna."

Weed and Cushing state: "In view therefore of the possible influence of these physical factors (alterations in capillary pressure due to changes in arterial and venous pressure produced by the injection, and the possibility of fluid displacement by the accumulation of venous blood) on the discharge of cerebrospinal fluid, it is hazardous to assume that certain agents stimulate the secretion of the choroid plexuses, merely because of an observed increase in flow from a cannula."

Dandy and Blackfan (24) describe a series of experiments in which they attempted to increase the fluid outflow from a cannula inserted into the canal through the atlas. They state: "The most striking and uniform result obtained . . . followed temporary compression of the jugular veins. Except in one instance there was always a marked and instantaneous increase of cerebrospinal fluid following jugular compression. We believe this can be explained only by an increased production of fluid."

*Experimental:* A study of the influence of the venous pressure can be carried out almost uncomplicated by alterations in the arterial pressure. This can best be done by ligation of the jugular veins. Ligation of the internal jugulars alone in most animals produces little effect, either upon the venous or the fluid pressure. In a very small percentage of cases a rather marked rise in the venous and the fluid pressure will follow such a ligation. (See table 6.) This is of course in keeping with the findings of Gärtner and Wagner (25). Unless otherwise specified, the term "ligation of the jugular" means ligation of the external ones only. The effect of the ligation of these veins upon the arterial and fluid pressures can best be shown by the use of tables. (See tables 6 and 7 and figs. 3, A, B and C, and 4).

TABLE 6

*Effect on the various pressures of ligation of internal and external jugulars successively and simultaneously*

		GENERAL ARTERIAL				TORCULAR VENOUS				C. S. FLUID			
		B.	D.	A.	Rise or fall	B.	D.	A.	Rise	B.	D.	A.	Rise
226	a	130	128	130	-2	208	226	207	18	90	93	84	3
	b	124	122	130	-2	201	230	209	29	81	101	78	20
	c	130	128	134	-2	201	237	188	36	75	99	72	24
279	b	162	154	160	-8	108	120	108	12	166	175	165	9
	a	160	160	160	0	108	240	108	132	160	241	166	81
	c	162	150	160	-12	108	280	104	182	165	267	160	102

TABLE 7

*Effect on the arterial and fluid pressures of the ligation of the jugular veins*

TRACING	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			C. S. FLUID PRESSURE		
	B.	D.	A.	B.	D.	A.	B.	D.	A.	B.	D.	A.
153	158	150	160	120	118	120	601	706	625	141	237	129
154	118	108	120	90	88	92	354	412	353	113	191	104
155	152	142	130	114	112	102	425	796	496	189	321	195
156	a	130	116	122	102	100	496	579	479	195	250	168
	b	122	116	112	100	98	484	560	529	164	210	145
158	114	108	118	88	86	90	356	477	357	122	191	112
160	156	150	158	132	140	142	58	271	79	60	195	65
161	120	110	120				150	420	124	201	403*	193
152	a	176	166	140	146	140	118	401*	217	214	321	229
	b	104	104	110	84	84	86	166	92	264	317	288
	c	94	94	102	80	82	66	143	72	109	161	105
	d	42	42	46			38	70	39	69	90	75
171a	142	138	140				84	125	88	56	84	54
197	148	150	162				150	324	165	99	213	120
200	158	150	158				142	295	155	108	195	108
226b	124	122	130				201	230†	209	81	101†	78
279a	160	160	160				108	240	108	160	241	166
158a	130	134	128	112	120	112	262	504	290	211	393	220
Average...	130.4	125.5	128.6	106.1	106.1	104.7	232.1	371.7	248.7	142.0	228.5	141.8

\* Greatest change from normal.

† Least change from normal.



Table 7 shows clearly that a rise in the venous pressure produced by the ligation of the jugulars is accompanied in every case by a rise in the pressure in the fluid. The rise in the latter is not so great as in the former, and these differences vary in the different animals, but the rise in the venous pressure, if great, is accompanied by a pronounced rise in the fluid pressure; if small, by a small rise in the fluid pressure. It is also to be noted that the changes are diphasic. The venous and fluid pressures rise and fall almost, in the best experiments absolutely,

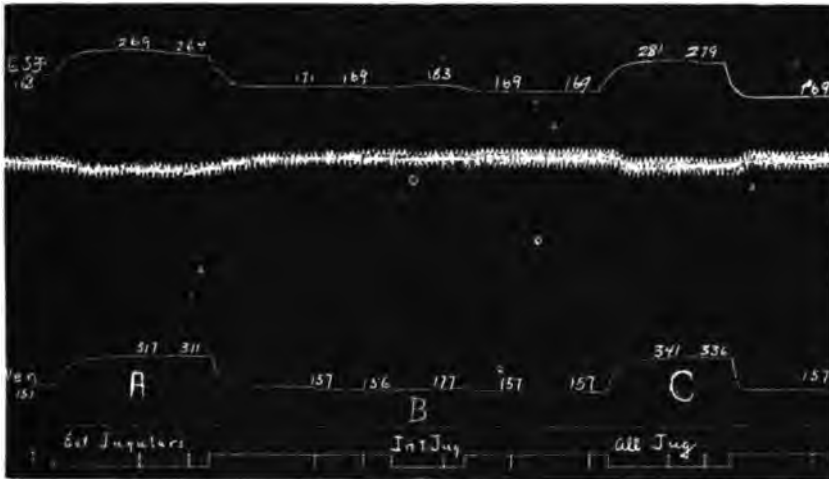


Fig. 3. This figure shows the differences in the reaction of a dog to ligation of the internal and external jugulars. It shows: *a*, the greater effectiveness of ligation of the external jugulars than ligation of the internal jugulars in raising venous and fluid pressures; *b*, the striking similarity in the curves of venous and fluid pressure, both as regards the time relation and the shape of the curves. The numbers represent millimeters of a solution with a specific gravity of 1.006. In this and in later tracings, *V* designates the torcular venous, *F* the C. S. fluid pressure.

synchronously. As can be noted from the figure, there is tendency for the fluid rise to lag behind the venous rise. The same holds true for the fall, a phenomenon which has an easy explanation in the greater complication of the system of canals through which the fluid must pass both in leaving and in reëntering the canal. The amount of delay varies widely between different animals. It is also to be noted from figure 4 that the height of the rise following ligation of the veins is dependent largely upon the height of the arterial pressure. If the latter

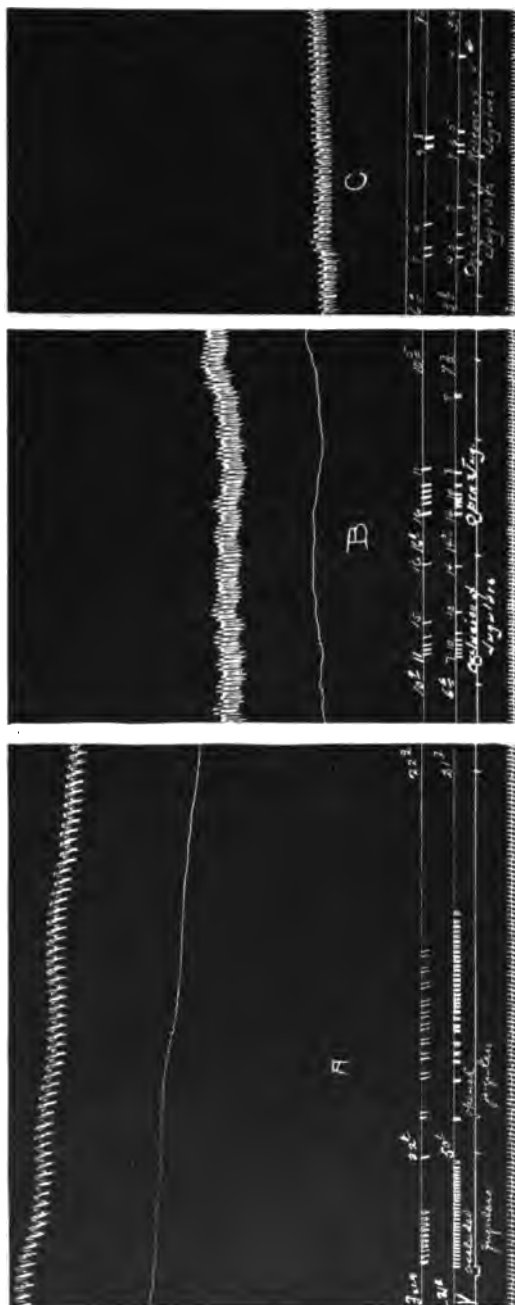


Fig. 4. This figure shows the variation in the result upon venous and fluid pressure of occlusion of the external jugular veins: A shows the result early in the experiment; B, the result 1 hour and 15 minutes later; C, the result 2 hours and 20 minutes later.

is high, occlusion of the veins produces a marked rise in the venous and fluid pressures; if low, ligation of the veins produces a small effect. This can be seen readily by reference to figure 4. In this figure we have the effect of three ligations in the same animal. The first was early in the experiment with a high blood pressure. It produced a great rise both in the venous and the fluid pressures. The second was in the middle of the experiment, the pressure had fallen in the artery, and the effect of the ligation of the vein was to raise the pressure in the sinus

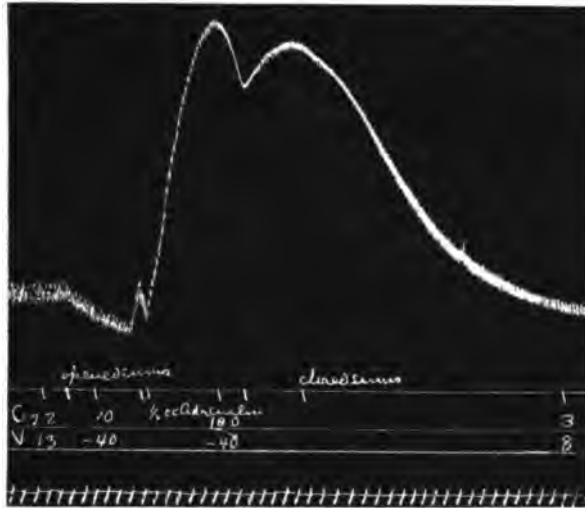


Fig. 5. This figure shows that when the sinus is opened so that the torcular pressure falls from 13 to  $-40$  mm. (53 mm.), the fluid pressure falls from 22 to  $-10$  mm. (32 mm.). The remainder of the tracing was a study on another phase of the subject, and is given only to show that the venous pressure rose to 8, the fluid pressure to 3 mm. after the sinus was closed and the effect of adrenalin had passed off.

and the fluid considerably but not nearly so much as in the first ligation. The third was at the end of the experiment, the arterial pressure was low and the effect on venous and fluid pressures was slight, almost negligible.

Further, lowering the normal venous pressures in the skull by opening the sinus results in a fall of the fluid pressure. (See fig. 5). Table 1 and figure 1 show that cerebral venous and general venous do not vary to the same degree or even in the same direction.

Since a rise in venous pressure always produces a rise in fluid pressure, and a fall in venous pressure always produces a fall in fluid pressure, it is self-evident that in any experiment the cerebral venous pressure must be measured *during the experiment* to eliminate the possibility of a venous pressure change before increased formation or absorption can be *proved*.

A study of the influence of torcular venous pressure upon the pressure of the cerebrospinal fluid makes it evident that any experiment involving the use of a drug, or any physiological manipulation which has the least possibility of producing changes in the venous pressure, is worthless unless the venous pressure in the sinus, not in the general circulation, is measured as well as the arterial and fluid pressures. The marked influence of venous upon the fluid pressure is so easily established, and its significance is so unmistakable, that it is surprising that any recent worker would neglect the measurement of this important factor in any experiment from which he intends to draw conclusions regarding the nature of the change in the fluid pressure and the fluid formation. If limited to the measurement of one of these besides the cerebrospinal fluid pressure he would do well to measure the venous pressure rather than the arterial, because of the relatively greater importance of the former than the latter in the determination of the fluid pressure. The fact that Frazier and Peet, Dandy and Blackfan, and Weed and Cushing in their work failed to record this pressure in a single one of their experiments, and that Dixon and Halliburton (12) in their work on the choroid, measured arterial and venous pressures in one animal or experiment, and arterial and fluid pressures in another, and took it for granted that the behavior of the venous pressure was the same in both cases—when such was not necessarily the case at all—detracts markedly from the value of their work.

Let us take up more in detail the criticism of the experiments found in the literature. We believe that the authors mentioned failed to measure the venous pressure simultaneously with other pressures, because they underestimated its influence on the fluid pressure. Dixon and Halliburton say: "The rise in C. S. pressure is only about one-quarter that which occurs in the torcula . . . when the two pressures are low the passive effects of increasing the venous pressure is negligible." Thus these authors while admitting that increasing the venous pressure passively increases the fluid pressure, do not find the increase in the latter to any degree comparable to the increase in the former. In our work, making use of a manometer with a fine bore, as

can be seen from the table, the changes in pressure in the venous and fluid systems are much more nearly equal than in the few experiments cited by Dixon and Halliburton. We are convinced that if we could measure the fluid pressure at the crest of the venous rise as accurately as we can at the bottom without permitting the escape of fluid into the manometer tube, the changes in fluid pressure would almost if not quite equal the changes in venous pressure. Their error lies in the fact that they used a piece of apparatus not adapted to the needs of the experiment, with the result that they did not measure the pressure in the normal intact animal, but the pressure change in an animal with the pressure reduced by the withdrawal of an amount of fluid necessary to fill the tube of the manometer to the required level. They failed to note also that if venous pressure is low the arterial pressure may be relatively high, and that when this is the case obstruction of the venous outflow or ligation of the veins is followed by a great rise both in the venous and fluid pressures, for it is the "highness" of the arterial pressure, not, as they state, the "lowness" of the venous pressure which determines the amount of rise in the venous pressure following occlusion and, therefore, the amount of rise in the fluid pressure. (See fig. 4. Compare also the results in tracing 152 a, with 152 d, table 7).

Nor can we concur in the statement made by the authors that: "Perhaps the most remarkable feature of the cerebrospinal fluid viewed from the physical side is the fact that relatively small circulatory changes as determined by the general arterial and venous pressures may cause large changes in the cerebral venous pressure, but have little effect on the cerebrospinal fluid pressure." As we have already said, the general venous and the torcular venous are practically independent of each other, they vary in the same or in opposite directions as is shown fully in table 1 and in figure 1. But in the measurements shown in the table, in only one case was the general venous higher than the torcular venous. We used the brachial vein in preference to the right heart because of the fact that the technic in using the former is less cumbersome, and the balloon in the auricle must dam the blood back into the great veins to some extent, and what is more dangerous, to a varying extent. The fluid pressure nearly always—in fifty of fifty-eight cases—varies in the same direction as the cerebral venous pressure, and in some degree proportionally.

Further we observe, as they have done, great changes in the torcular venous pressure without a corresponding change either in general ar-

terial or venous pressures. Extreme care must be observed in this work; a rise in general venous pressure usually gives a rise in the torcular pressure; movements of the head, perhaps shifting the muzzle only a few inches will raise the torcular pressure 30 or 40 mm. probably by occluding the jugular veins in the neck. The observation was made long ago that shifting the head would raise the fluid pressure (16). The explanation given by this writer was that the lateral movements of the spine narrowed the space available for the fluid and thus increased the pressure of the fluid in the canal. The increase in venous pressure invariably increases the pressure of the fluid. Placing the hand on the neck or on the abdomen has the same effect. In the experiments which we performed and considered free from error, any rise in the venous pressure in the torcula was always accompanied by a rise in the fluid pressure. Therefore, the last statement in the quotation above is an error, for as can be seen from our experiments on venous occlusions, the cerebral venous pressure invariably *does* influence the fluid pressure directly. If this is true for venous pressure raised passively, it is true also for the pressure rises in venous blood, whether caused by drugs or other agencies.

From the conclusions of Frazier and Peet regarding the influence of the venous pressure on the fluid, it is evident that they also underestimate the importance of this factor. A rise of 40 mm. in fluid pressure is not as much as is to be expected on ligation of the jugular vein if the animal is in good condition. The rises in our experiments averaged 86 mm. We do not know the diameter of the manometer tube they used, so cannot judge whether the error is in the apparatus or is to be ascribed to some other source. However, in our experiments, animals long under ether anesthesia showed practically no rise in the venous pressure on ligation of the veins, because the arterial pressure was too low. This may have been the cause of the small rise they record; or, morphine-urethane anesthesia pushed too far would produce the same results. The fact remains that ligations such as they describe, carried out in fresh animals under a proper degree of anesthesia, show the results given in table 7, which records the effects on the venous and fluid pressure of the ligation of the jugulars in eighteen animals. It will be seen that the greatest change in venous pressure was in no. 152 a, where the venous pressure rose 383 mm. of sodium carbonate, while the fluid pressure rose 132 mm. of NaCl. The greatest change in fluid pressure was in no. 161, where while venous pressure rose 270 mm. of sodium carbonate, fluid pressure rose 202 mm. NaCl. The smallest rise was in no. 226 a,

where the venous rise was 18 mm., the fluid rise 3 mm. The average for the whole series was arterial rise  $-4.9$ ; venous 139.6, fluid 86.5. These results show conclusively that the figures reported by Frazier and Peet are too low, hence the importance of the venous pressure in the experiments—a pressure which they mention, but do not record—is enormously increased.

These tables substantiate the statement made by Hill long ago, that an increase in the venous pressure, uncomplicated by any marked change in the arterial pressure, is accompanied by an increase in the cerebrospinal fluid pressure; while a fall in the venous pressure is accompanied by a fall in the cerebrospinal fluid pressure. This proves clearly that in those cases where venous pressure was not recorded the writers do not have all the facts regarding the forces responsible for the changes in the pressure and the outflow of the fluid. This one fact eliminates a large number of recent experiments from consideration. Dandy and Blackfan apparently were not aware of the fact that the outflow of the fluid was only half of the total change in the fluid which occurs on ligation and release of the veins, for under suitable conditions of experimentation the fluid would have returned to the skull in an amount equal to the amount which escaped. They would not have drawn the conclusions they did if they had known that the changes are reversible. The fact which they consider of so much importance, that the original rate of outflow from the cannula was reestablished after the release of the veins, is of no great significance, for with the release of the veins and the reduction of the venous pressure in the skull, fluid from some other place in the subarachnoid space was released and flowed out of the needle. This explains why repeated ligations and release of the jugulars produced repeated increases in the outflow, as well as the observation that the original rate of outflow was reestablished. According to our own observations on this point repeated ligations of the veins become progressively less effective in producing fluid.

Having established the fact that the changes in the venous and fluid pressures are in the same direction, more or less equal in amount, and reversible, it is necessary to inquire into the nature and mechanism of the changes. We believe that one of three explanations is the correct one:

1. Increased formation [due *a*, to increased capillary tension; *b*, to increased secretion due to asphyxia,] perhaps complicated by impaired absorption.

2. Increased venous pressure with the accumulation of venous blood in the sinuses and the displacement of fluid; an increased accumulation of venous blood without an increased pressure is possible.

3. Simultaneous but independent rises in fluid and venous pressures.

1. Ligation of the veins increases the capillary pressure in the skull. Because the venous pressure is increased without much reduction in the arterial pressure, filtration is favored, if that be the mechanism of the formation of the fluid. Frazier and Peet (14, p. 484) believe this factor important for pressures over 190 mm. of water in the veins. Weed and Cushing (8, p. 100) say "venous pressures . . . may exert considerable effect upon the secretory pressure through alteration in the capillary tension for, as Starling has demonstrated, capillary pressure follows more closely upon alteration in venous pressure than upon far greater changes in the arterial tonus." Ligation of the veins produces venous stasis, and thus asphyxia may stimulate the cells to increased activity, if secretion is the mechanism of the formation of the fluid. The rôle of asphyxia as a stimulus to the cells of the choroid has been strongly emphasized by recent writers (12). In addition to this, since it is evident that the absorption of the fluid is by way of the veins rather than by the lymphatics, ligation of the veins should prevent absorption and thus favor the accumulation of the fluid. Hence the fluid pressure may rise, not only because more fluid is formed, but because less is absorbed from the canal. On this point Frazier and Peet (p. 274) say: "The more or less constant rise in pressure when the venous circulation is interrupted confirms the hypothesis that the absorption of cerebrospinal fluid takes place chiefly through the venous channel. We can show that neither filtration nor secretion need be assumed to explain the facts."

2. The second explanation of the rise in the fluid pressure on ligation of the jugulars is that it is due to the increase in the venous pressure, which permits the accumulation of the venous blood in the sinuses, and displaces the fluid from its usual place in the canal.

In favor of the second we have the observation that the changes in the two pressures are synchronous. And not only are the changes parallel but they are to some degree proportional. (See table 8 and fig. 4). The rise in the fluid pressure is 61 per cent of the rise in the venous pressure, and the fall is 69 per cent of the fall in the venous pressure. This parallelism is confirmed in the work of Dixon and Halliburton. They do not confirm our findings as regards the amount of change: "The rise of the C. S. pressure is one-quarter that which occurs in the



torcula." The explanation of the differences between their observations and our own has already been fully discussed in the section on the influence of the size of the manometer tubes. The failure of Dixon and Halliburton to notice that the venous and the fluid pressures vary much more nearly to the same degree, led them to the erroneous conclusion that "fluid pressure is an independent pressure and the factors determining its height are dependent on the rate of secretion and absorp-

TABLE 8

*This table shows the effects on the arterial and venous pressures of increasing the cerebrospinal fluid pressure by small amounts (98 to 202 mm. of NaCl Sp. Gr. 1088)*

TRACINGS	ARTERIAL			TORCULAR			CEREBROSPINAL FLUID PRESSURE			REMARKS
	B	D	A	B	D	A	B	D	A	
188	150	148	146	120	123	122	93	191	182	Fluid held in canal
189	130	120	120	113	116	135	92	280	127	Drew out 2.5 cc.
190	142	144	142	104	105	105	76	274	104	Drew out 2.5 cc.
191	140	140	140	96	99	103	71	170	76	Drew out 2.5 cc.
192	124	120	106	145	112	125	123	325	137	None drawn off
193	90	90	84	84	92	88	97	254	230	None drawn off
194	92	92	84	61	61	61	91	257	145	Fluid allowed to escape
195	114	146	114	75	63	84	65	260	68	Fluid allowed to escape
196	132	124	118	84	76	66	68	258	183	Fluid allowed to escape
197	106	106	98	54	59	59	88	254	185	Fluid allowed to escape
229a	108	168	112	75	75	63	72	264	65	
229b	112	146	112	63	68	76	65	260	68	
229c	136	124	116	76	76	54	68	258	86	
229d	104	104	98	54	59	59	88	254	185	
231a	96	100	92	63	62	55	68	331	71	
231b	88	96	88	53	64	52	64	331	73	
232	84	96	84	54	67	61	61	324	74	
233	78	92	64	51	56	52	66	343	81	
234	66	60	58	64	75	79	111	264	95	

tion." That this statement is not warranted by experiment is evident from the tables cited and figures shown above: where it is shown clearly that under suitable conditions of measurement, the venous and fluid pressures run a remarkably parallel course. They vary in the same direction too often to be independent of each other. Modifying the venous pressure changes the fluid pressure in the same direction. It is clear from these facts that one of the factors determining the fluid pressure in the animal is the venous pressure in the skull. If the pressure

varies in the experimental animal under the influence of changes in the venous pressure, there is no reason for assuming that it does not do so in the intact animal.

In order to prove that the occlusion of the jugulars is not accompanied by the formation of an increased amount of fluid, the following experiment was carried out:

Small, black, male cur dog, weight 11 kilos. Animal prepared by the usual method as described above. Ether by constant insufflation. With all the pressures constant 1 cc. of NaCl was injected into the dural canal. The arterial pressure was unchanged at 124 mm., venous pressure was *unchanged* at 144 mm., fluid pressure *rose* from 120 mm. to 230 mm., as recorded by our 1 mm. manometer tube. The time required for a return approximately to the normal was 6 minutes and the point reached was 126 mm. After the return of the pressure to the normal, the fluid pressure was raised to the same degree by ligation of the veins. In this case *both venous and fluid pressure rose*, the former from 146 to 250 mm., the latter from 126 to 225 mm. Release of the veins was followed by a rapid return of both pressures to the normal levels, in 11 seconds.

This experiment, fully substantiated by numerous others, shows conclusively that the time required to reduce the fluid pressure by the absorption of a sufficient amount of fluid necessary to produce a rise of about 120 mm. in the fluid pressure is enormously longer—about thirty-three times as long—than the time required to reduce an equal increase in the fluid pressure produced by ligation of the veins. Further, the former rise is accompanied by *no change* in the venous pressure, the latter is accompanied by *an increase* in the venous pressure. For these reasons it is perfectly clear that the rises in the two cases must be due to fundamentally different causes. Since in the first experiment, where we *know* there was an increase in the fluid 6 minutes were required for readjustment, while the last experiment where the pressure rose to the same degree required only 11 seconds for readjustment, where new formation was assumed but not proved, we believe that we are justified in stating that ligation of the jugulars does not cause "increased production of fluid," nor so far as our experiments are concerned is there any evidence whatever that there is delayed absorption of fluid. If any increase in the fluid in the canal resulted from the ligation of the veins, a longer period than 11 seconds would be required for readjustment after the release of the veins, for a longer period is required where the fluid is introduced by means of a hypodermic. This rapid readjustment can be seen graphically recorded in figure 3.

The third explanation that the rise in pressure is simultaneous but independent is possible but improbable in the highest degree.

Table 7 establishes the following facts: The ligation of the jugulars—taking an average of eighteen experiments—raises the venous pressure 139.6 mm., the fluid pressure 86.5 mm. Releasing the jugulars lowers the venous pressure 124.0 mm., the fluid pressure 86.7 mm. The changes in the venous and fluid pressures are synchronous both in a positive and a negative direction. As will be noted, both pressures returned approximately to the starting point immediately after the veins were released, a point of theoretical importance.

The conclusions we draw are the following: Raising the venous pressure raises the fluid pressure, lowering the venous pressure lowers the fluid pressure. The changes are not equal, but are more nearly equal in our experiments than in those reported by our predecessors, and we believe they would be almost exactly equal if a perfect method for measuring were devised, one by which the measurement is taken without withdrawing fluid from, or adding fluid to the canal. The measurement of venous pressure is absolutely necessary in order to rule out mechanical changes due to changes in venous pressure.

#### *4. The influence on the venous pressure of altering the cerebrospinal fluid pressure*

In the last section it was shown that raising the venous pressure increases the fluid pressure, and lowering the venous pressure lowers the fluid pressure. Let us now consider what influence raising or lowering the fluid pressure has on the venous pressure. Our results are shown in table 8, and typical graphs in figures 6 and 7.

From the table and the figure it is evident that *increasing the cerebrospinal fluid pressure does not increase the venous pressure*, so long as the rise in the fluid pressure does not produce a rise in the arterial pressure. If the rise in the fluid pressure is sufficient to produce a rise in the arterial pressure, then the venous pressure may rise with it, as in figure 6, but such is not always the case. (See fig. 7). These results are exactly contrary to those reported by Dixon and Halliburton. In figure 9 (p. 139) these authors show a marked rise in venous pressure on raising the fluid pressure by 100 mm. of saline. That these authors do not find such a rise in venous pressure consistently is evident from their own tracings: Compare figure 9 (p. 139) with figure 15 (p. 147). In the latter experiment the rise in the fluid pressure was from 60 to 160 mm.—exactly 100 mm. rise—as in the other experiment, but the rise here was accompanied in the early period by no effect on the venous pres-

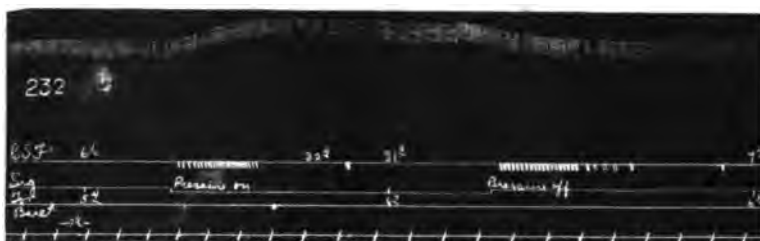


Fig. 6. This figure shows the effect on torcular venous and arterial pressures of raising the cerebrospinal pressure by 263 mm. of sodium chloride solution (from 61 to 324). Note that the arterial pressure rose slightly, and venous pressure rose slightly (13 mm.), as is to be expected under the slight rise in the arterial pressure.

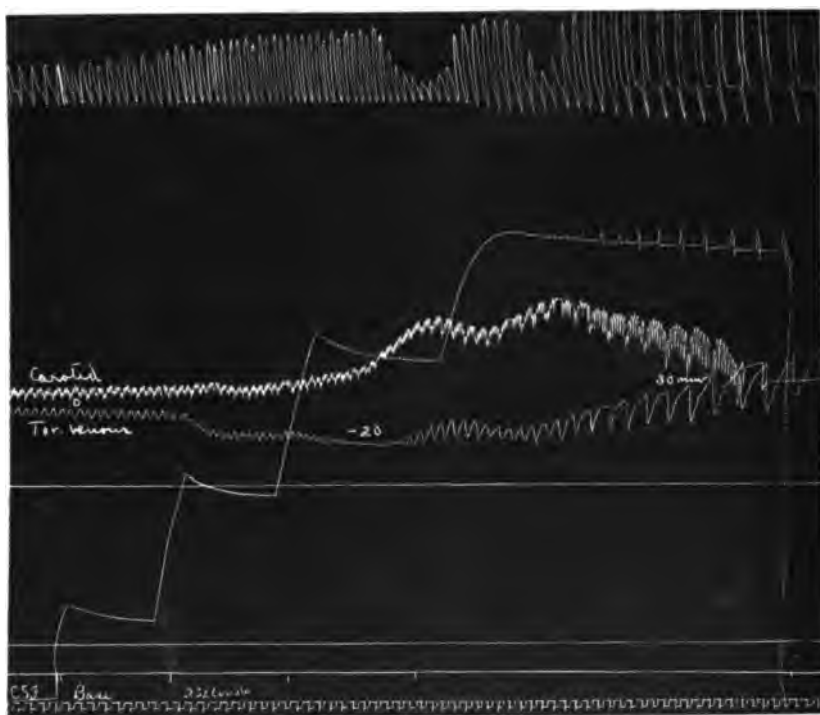


Fig. 7. This figure shows the results upon arterial and torcular venous pressure of increasing the fluid pressure. The pressure of the fluid is recorded graphically in millimeters of mercury. It is to be noted that at no time was the cerebral venous pressure increased. Note also that as the fluid pressure approaches that of the artery, the cardiac oscillations become more marked upon the curve traced by the fluid manometer.

sure, other than one synchronous with, and in the direction to be expected from a change in the arterial pressure. If their observations regarding the influence of the fluid pressure upon the venous pressure were correct the venous pressure should have risen markedly *early* in the experiment at the time the fluid pressure rose, but venous pressure did not rise. The same criticism holds to a less degree for the experiment shown in figure 14 (p. 146). The fact that the rise in the fluid pressure was produced by different means in the different experiments does not alter the results, to be expected from such a rise in the fluid

TABLE 9

*Effects on the various pressures of the withdrawal of blood and its reinjection.  
Hemorrhage and transfusion*

TRACING	AMOUNT WITH- DRAWN AND REINJECTED	ARTERIAL			CIRCLE OF WILLIS			TORCULAR			CEREBROSPINAL FLUID		
		B	D	A	B	D	A	B	D	A	B	D	A
	cc.												
159a	100	118	64	104	90	52	78	356	171	364	112	49	122
159b	100	102	52	70	76	44	52	363	236	229	122	45	91
163	100	128	104	128				53	16	42	18	-16	6
164	100	132	84	140				60	32	56	15	6	20
165a	100	120	76	110				40	9	38	6	-23	0
165b	100	120	64	106				66	65	64	100	3	112
166	100	120	82	126				72	18	57	36	-12	20
174a	100	108	80	112	72	62	74	205	138	202	98	65	108
175b	100	114	90	118				208	168	205	110	75	116
205	100	152	132	158				146	123	138	97	78	108
206	100	154	130	156				138	126	133	104	76	107
207	200	146	104	144	90	72	94	138	110	146	97	49	103
Averages		126.1	88.5	122.6	82	57.5	74.5	153.7	101.0	139.5	76.2	32.9	76.1

pressure, for in our opinion the rise cannot be due to anything other than a mechanical change, and even if the fluid were increased, if their conclusions are correct increased fluid pressure must raise the venous pressure at the same time.

In the experiment cited in table 9 it is to be noticed that no rise such as they report as the usual result ever appeared. This is true whether the pressure be applied at the fourth ventricle through a second needle inserted near the trocar through which the fluid pressure is measured, or in the parietal region through a tube screwed into the skull, after

trephining and incising the dura. In no case did the venous pressure change to any degree under the influence of moderate increase in fluid, unless there was a synchronous and parallel change in the arterial pressure in amount sufficient and in direction correct to account for the venous pressure change. Further, it was noted in experiments performed for another phase of the problem that injections made into the third ventricle by transcallosal puncture raised the fluid, but not the venous pressure. We noted the increase in the venous pressure on increasing the fluid pressure in one animal only, and in that animal a post-mortem examination revealed a communication between the sinus at the torcula and the subarachnoid space, with blood in the fluid. In this case injection either in the parietal region or into the fourth ventricle increased the pressure in the vein. From these facts we are forced to the conclusion that figure 9 shown in Dixon and Halliburton's work may have been made upon an animal with such a communication between the sinus and the subarachnoid space. In our nineteen experiments made on six dogs, where no communication existed *increasing the cerebrospinal fluid pressure DID NOT increase the venous pressure in the sinus*. In four experiments on one animal, where such a communication existed, raising the fluid pressure raised the venous pressure, but not to the same degree as the fluid pressure. This is proof for the statement made earlier in the paper, that the fact that the venous and fluid pressures were not the same is no proof that no communication between the sinus and subarachnoid exists.

A careful consideration of the structures within the skull and of their arrangement would warrant the above findings. The pressure of the venous blood lying within the flexible sinuses must of necessity exert much influence upon the fluid, if the latter surrounds the sinuses in normal amounts. On the other hand the fluid cannot exert much influence upon the venous pressure, for as soon as the pressure in the subarachnoid space increases appreciably above that in the venous sinuses, the latter will collapse, forcing the blood onward toward the heart. Any further increase in the pressure will then be exerted upon the skull, not upon the venous blood in the sinuses.

The use of higher pressures always results in alteration of the arterial pressure and a change in the venous pressure, sometimes raising, sometimes lowering it.

Lowering the fluid pressure by drainage does not affect the venous pressure. If lowering the fluid pressure lowers the venous pressure, and a normal venous pressure is necessary for the normal formation of

the fluid, then none of the experiments reported by Dixon and Halliburton in their first paper can be correct, for they used the outflow method for estimating the influence of extracts of the choroid and other substances upon the fluid, a method which reduced the fluid pressure, and if they are correct must have reduced the venous pressure. The same criticism holds for all experiments in which the outflow method was employed. However, reducing the fluid pressure does not reduce the venous pressure in the torcula.

The conclusions we have reached are the following: Raising the fluid pressure by moderate amounts (100 to 200 mm. of NaCl solutions sp. gr. 1088) *does not* affect the venous pressure unless the arterial pressure is affected. Lowering the fluid pressure does not affect the venous pressure. The conclusions of Dixon and Halliburton are not in keeping with our results, and may have been based on observations on an animal with a communication between the sinus and the subarachnoid space. As shown in table 9, in nineteen experiments on six animals in which no such communication existed as proved by the post mortem, raising the pressure moderately produced no rise in the venous pressure. In four experiments where such a rise was seen, a communication between the sinus and the subarachnoid space was found at autopsy. We are dealing here with a question of fact, and confirmatory evidence from other workers is needed. However, we are sure that evidence in support of our own results will be forthcoming.

In figure 7 we show an experiment where higher pressures were used. The pressures in this case are recorded in millimeters of mercury and the venous pressure fell. This gives purely objective proof that Dixon and Halliburton (p. 139), are wrong in their conclusion that "Such a rise (in C. S. pressure) always produces a passive increase in cerebral venous pressure." This experiment gives the facts for low as well as high pressures. This same tracing gives the proof also that Frazier and Peet (p. 272) are incorrect, at least so far as cardiac oscillations are concerned, in their statement: "Sufficient intracranial pressure can easily be made to arrest these respiratory and cardiac oscillations. . . . When a column of water was introduced under pressure only slightly in excess of the venous pressure the oscillations were arrested." That Frazier and Peet are not correct is brought out clearly in our graph. The oscillations in the mercury manometer become more nearly equal to those of the blood pressure manometer, the nearer the pressure in the subdural space approaches the intravascular diastolic arterial pressure. This is exactly what is to be

expected, for the skull, brain and the intracranial blood vessels reproduce almost exactly the conditions obtaining in the arm band of the blood pressure machine when the diastolic blood pressure is being measured. The undilatable cranium represents the arm band, the brain the muscles of the arm, and the layer of blood vessels the pulsating brachial artery. If the oscillations in the air of the expanded arm band increase the more nearly the pressure in the band approaches that of the blood vessel, then the oscillations of the fluid must increase the more nearly the pressure equals that of the diastole in the pulsating vessels.

Since venous pressure can be shown to affect the fluid pressure directly, and since the fluid pressure can be shown not to affect the venous pressure directly, it follows that all other factors remaining constant, e.g., the arterial pressure, secretory pressure, etc., then if both venous and fluid pressures rise at the same time, then the rise in the venous pressure caused the rise in the fluid pressure, and not the rise in the fluid pressure the rise in the venous pressure, it is theoretically possible, then, if secretagogues for the fluid exist, to increase the volume of the fluid in the canal by the action of the substance of the secreting cells to such a degree as to produce a rise in the fluid pressure independent of a rise in the arterial and venous pressures, a change which would be impossible if Dixon and Halliburton were correct, for as the fluid pressure rose it would force the venous pressure to rise with it. Further comment on this phase will follow later under the action of drugs.

*5. The effects of altering both arterial and venous pressures in the same direction*

Having studied the effects of alterations in venous and fluid pressures with little change in the arterial pressure, it becomes necessary to study the effects on the fluid pressure of alteration in the arterial and venous pressures when these vary in the same direction. We will study first the effect of reducing these pressures.

*Literature:* On this point we find only the work of Dixon and Halliburton (12) who summarize (p. 142) their observations as follows: "If the arterial and venous pressures are lowered, as for example by bleeding, the drop in C. S. pressure is relatively small."

*Experimental: a. Hemorrhage and transfusion.* We carefully repeated these experiments with the modification that the blood was drawn



from the femoral artery into a large pipette and re-injected into the femoral vein before coagulation could occur. Table 9 shows the results of twelve such experiments. As may be seen from the averages, all three pressures fall on the withdrawal and rise after the restoration of blood. As may be seen from figure 8, the fall in all the pressures is synchronous, as is also the rise. The readjustments are good: all the pressures remain on the average a little higher than they were originally. There is every reason for believing the change here to be of a purely mechanical nature, for there is no apparent reason for thinking them to be anything else. By no conceivable way can we see how hemorrhage and transfusion could have any but a purely mechanical effect by way of the vascular system. As will be seen from our experi-

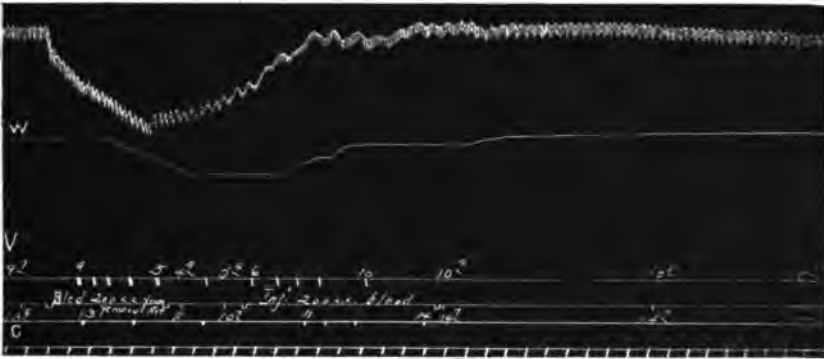


Fig. 8. This figure shows the effect of the withdrawal of 200 cc. of blood from the femoral artery, and its reinjection into the femoral vein.

ments, the average fall in torcular venous pressure was 52.7 mm., in fluid 43.3 mm., showing the fluid pressure fall to be 80 per cent of the fall in the venous pressure. This is much greater proportionally than the amount of fall noted by Dixon and Halliburton, who in a single experiment recorded show a fall in the fluid of exactly 10 per cent of the fall in the venous pressure. In the graph they show (p. 133) the venous pressure fell from 380 to 230, a fall of 150 mm. of 10 per cent citrate; the fluid pressure fell from 160 to 135, a fall of 25 mm. 10 per cent citrate. The explanation of the marked fall in our experiments as compared with those in the experiments of Dixon and Halliburton is found, of course, in the discussion of the influence of manometers of various sizes. There we found that with a wide manometer tube,

with a falling venous pressure, the fluid pressure would always remain higher than would be the case in the intact animal from overfilling the canal by the excessive amount of fluid in the manometer.

The authors named did not perform the reverse of this experiment; viz., the re-injection of the blood. As can be seen from table 9, re-injection of the blood raises all three pressures well up toward the original levels. Arterial pressure rises from 88.6 to 122.6; venous pressure rises from 101.0 to 139.5; and fluid pressure rises from 32.9 to 76.1. In this case in the average of twelve experiments the fluid rise is 110 per

TABLE 10

*Effect of ligation of arteries entering the circle of Willis*

TRAC- ING	VESSEL	ARTERIAL			CIRCLE OF WILLIS			TORCULAR			CEREBROSPINAL FLUID		
		B	D	A	B	D	A	B	D	A	B	D	A
177	Right caro- tid	108	92	118				145	69	139	46	25	54
178a		112	124	114				142	101	138	52	25	66
178b		112	80	120				138	110	148	64	5	82
279d		164	160	160				102	84	101	157	140	157
Average .....		124	114	128				131.7	91.0	131.5	79.7	51.2	89.7
179	Carotid and verte- brals	114	112	118				216	146	206	87	57	90
181c		118	70	120				235	215	243	85	53	66
181d		124	90	124				202	208	235	64	81	80
201		150	174	146	98	30	84	142	65	146	99	1	97
202		146	168	146	88	40	84	137	78	128	88	13	85
203		148	164	146	84	40	88	128	83	136	85	16	93
204		148	174	156	88	36	80	134	79	161	89	23	115
173b		134	160	142				74	58	72	42	21	24
Average .....		135.2	139.0	137.2	89.5	36.5	84.0	158.5	116.5	165.9	79.9	33.1	81.2

cent of the rise in venous pressure. This excessive rise is due undoubtedly to the piling up of fluid in the medullary region. It is evident that lowering venous and arterial pressure is accompanied by a proportional fall in the fluid pressure.

*b. Ligation of the vessels to the head.* Another easy way of producing decreased arterial pressure in the skull without much effect upon the general arterial pressure is to ligate the arteries forming the circle of Willis. As has been shown by Hill, ligation of the vertebrales and the carotids is not followed in the dog by an anemia sufficient to produce death, because of the anastomosis of the arteries of the trunk with

those of the spinal cord. Thus sufficient blood supply to the brain is afforded to support life. Experiments on this point are shown in table 10 and in figure 9.

As may be seen by the study of these reports, ligation of one or more of the three remaining arteries entering the circle of Willis (the left carotid being used for the blood pressure) is accompanied by a fall in the arterial, venous and cerebrospinal fluid pressures in the head, and a rise in the general arterial pressure.

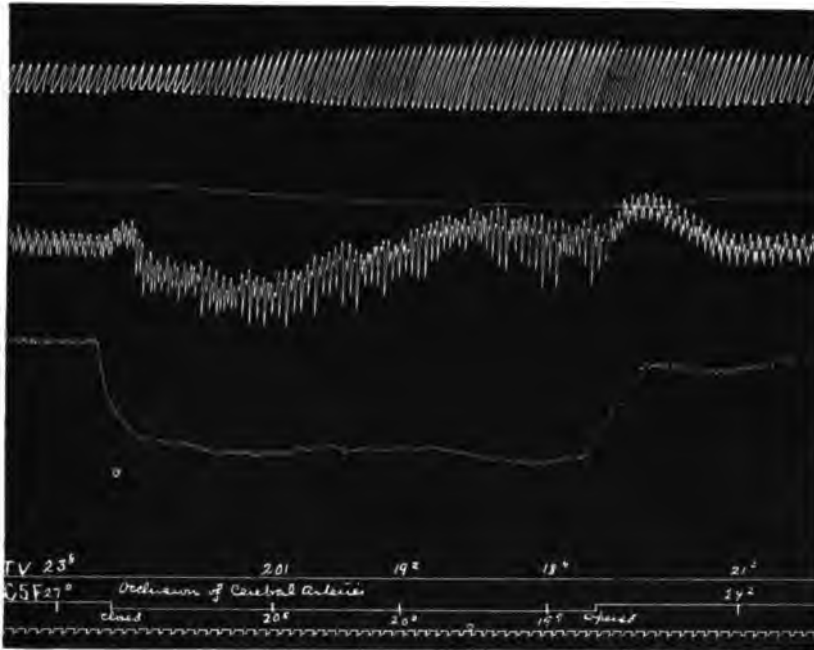


Fig. 9. This figure shows the effect on all the pressures of ligation of the arteries entering the circle of Willis.

In the manometer experiments ligation of the remaining carotid (right) produces an average fall of venous pressure of 41.7 mm. of sodium carbonate (1088), an average fall of fluid pressure of 31 mm. NaCl (1088), and an average fall of 8 mm. of mercury in the general arterial pressure. Ligation of the remaining carotid and both vertebrals resulted in an average fall in venous pressure of 42.0 mm., an average fall in fluid pressure amounting to 46.8 mm., a rise in general arterial pressure of 3.8 mm. of mercury, and a fall in the intracranial

arterial pressure of 53.0 mm. of mercury. Release of the carotid artery produced an average rise of 42 mm. in the venous pressure, of 38.5 mm. in the fluid pressure, and of 12 mm. Hg. in arterial pressure. Release of the carotid and vertebrals caused a rise of 47.5 mm. in the venous pressure, of 49.4 mm. in the fluid pressure, and a fall of 1.8 mm. in arterial pressure. These experiments clearly establish the fact that decreasing the arterial pressure to the brain decreases both venous and fluid pressures in the skull. Raising the arterial pressure raises the venous and fluid pressures in the skull. There is absolutely no reason for believing that the effects of the brief ligations are anything other than the effects of alterations in the arterial and venous pressures in the skull, reflected upon the fluid pressure. Experiments of this type do not give any evidence regarding new formation of the fluid although other writers have drawn different conclusions. A comparison of the pressure before and after ligation of the arteries shows an almost perfect readjustment to the original levels, a fact that speaks for mechanical alterations and readjustments. Only a few experiments of long duration were carried out. We have one in which the ligation of the carotid and vertebrals was maintained for 63 minutes. Pressure in the vein and fluid as well as in the circle of Willis remained low during the occlusion, and the general arterial pressure fell. On release the pressures all reached their original levels, except for the general arterial pressure, which remained low. We conclude, then, that ligation of the cerebral vessels produces only passive changes in the skull, characterized by a fall in the arterial, venous and fluid pressures in the cranial cavity, with an almost perfect readjustment when the vessels are released. This gives no evidence regarding the formation of the fluid, and does not give conclusive evidence that arterial pressure influences fluid pressure directly, for venous pressure fell simultaneously with the fall in arterial pressure.

*c. Ligation of the various trunk arteries.* We studied the effects of ligation of various arteries of the trunk on the fluid pressure, in an attempt to raise the blood pressure in the cranium above the normal by mechanical means. In both sets of experiments just cited, we are dealing with pressure falling below and then rising to the normal. As is so often the case in experiments of this kind, arterial pressure did not rise to any marked degree and hence the changes in other pressures are small. (See table 11, which shows the results of five such ligations. See also fig. 10).

As is apparent from the data in table 12, occlusion of the aorta or of the aorta and subclavians produces a slight rise in the arterial pressure accompanied by a slight rise in the venous and the fluid pressures. Release of these arteries produces a return of the pressures to almost exactly the original levels. These changes are purely mechanical: there is absolutely no reason for assuming either an increase or a decrease in fluid formation in such manipulation, the readjustments are almost perfect, and the changes are synchronous with and in the direction to be expected from the direction of the change in arterial pressure.

*d. Adrenalin.* A much more effective way of producing a rise in the arterial and venous pressure in the skull is the intravenous injection

TABLE 11  
*Effect of ligation and release of branches of the arterial tree*

TRAC- ING	VESSEL LIGATED	ARTERIAL			TORCULAR			CEREBROSPINAL FLUID		
		B	D	A	B	D	A	B	D	A
171b	Occlusion of aorta.....	138	132	140	83	75	68	50	46	47
172	Occlusion of aorta and subclavians.....	132	128	138	93	130	100	56	78	72
173a	Occlusion of aorta and subclavians.....	132	132	134	70	109	75	46	66	45
176a	Occlusion of aorta and subclavians.....	118	128	118	166	175	162	79	89	79
b	Occlusion of aorta and subclavians.....	116	124	110	164	170	156	62	68	53
Average .....		127.2	128.8	128.0	115.2	131.8	112.2	58.6	69.4	59.2

of adrenalin. This is especially true if the animal has been vagotomized or atropinized before the adrenalin is injected. When the blood pressure is raised by adrenalin there comes a synchronous rise in both venous and cerebrospinal fluid pressures. These rises in pressure, particularly that in the torcula, are so absolutely synchronous with the change in arterial pressure, that the conclusion that the rise in arterial pressure is the cause of the rise in the other pressures is inevitable. There is, as has already been stated, a tendency for the fluid pressure change to lag behind the arterial and venous change, so there comes a time, immediately after the end of the arterial and venous rise, when the arterial and venous pressures are stationary or falling at a time when the fluid pressure is still rising. This fact may seem to be

hard to correlate with the statement made above that the arterial and venous rise is the cause of the fluid pressure rise. Matters become clear, however, when the various straits in the fluid pathway are considered. The resistance offered by these straits slows the adjustment of the fluid to such a degree that the fluid pressure never records its true maximum for two reasons: first, the pressure recorded is the true fluid pressure minus the amount lost by the fall in arterial and venous pressures; second, to this error must be added the error of pressure lost due to the draining of a certain amount of fluid from

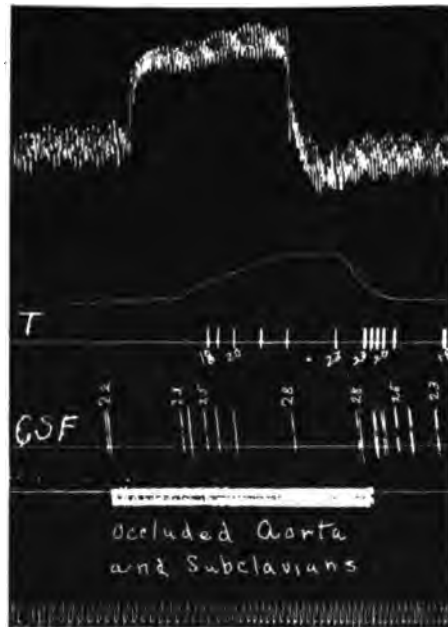


Fig. 10. This figure shows the effect on arterial, venous and fluid pressure of temporary ligation of the aorta and subclavians.

the canal into the manometer. The amount of this second error is modified by the bore of the manometer tube; it is large with a tube of wide bore and small with a tube of narrow bore. These two errors are always present and modify results to a considerable degree. Thus we are certain that if true fluid pressures were secured the pressure in the canal is considerably larger than those we record in our tables. In table 12 we record the results from eleven experiments. Many more were performed, but these show conclusively the results secured on this phase of the subject. (See fig. 11).

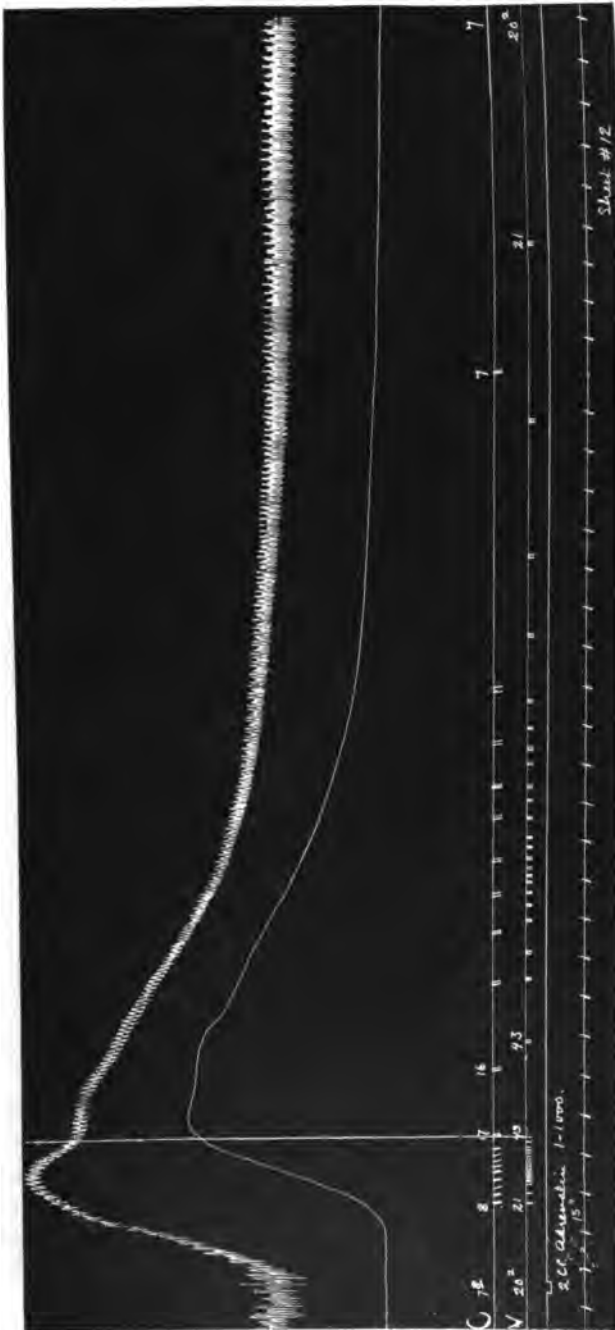


Fig. 11. This figure shows the effect on all of the pressures of raising the blood pressure by the intravenous injection of adrenalin. Note that fluid pressure, *C*, continued to rise an instant after venous pressure, *V*, had become stationary. Both venous and fluid pressures were still rising while arterial pressure was falling. Note that the readjustment is rapid and almost perfect. Compare this figure with figure 12 made on the same animal but after ligation of the jugulars.



Fig. 12. From the same animal as figure 11, and made immediately after 11. The jugulars were ligated and adrenalin was administered intravenously. Note that all the pressures are practically the same as those shown in figure 11. In this case ligation of the jugulars produced no rise in the venous and fluid pressures.



It may be assumed that the rise and fall in venous and fluid pressure *may* be due to something other than the rise and fall in arterial pressure. For example, if the fluid should accumulate rapidly in the animal with the cranium intact as Spina observed in the animal with the calvarium removed—the rise in fluid pressure might be due to increased transudation. Then, if Dixon and Halliburton are correct in their contention that raising the fluid pressure raises the venous pressure, this increase in fluid pressure would produce an increase in venous pressure, which would persist until the fluid was absorbed—a process taking place, according to all modern workers, along the venous channels. Ligation of the jugulars must then produce ideal condition for transudation for it would increase capillary pressure and this would be markedly augmented by the action of adrenalin forcing the arterial pressure to a high level. The fluid then being formed could not be absorbed because of the failure of outflow of blood through the jugulars; and thus if Dixon and Halliburton are correct in their contention, the fall of fluid and venous pressure would of necessity be delayed in the case where the jugular is ligated. (See fig. 12). Comparison of the two figures, 11 and 12, shows that allowing for difference in the arterial curves, the return to normal is as rapid in the case where the jugular is ligated as in the case with the jugular open.

From the data shown in table 12 it is evident that a rise in arterial pressure caused by adrenalin is accompanied by an enormous rise in the venous and fluid pressures inside the skull. This venous pressure rise is absolutely synchronous with the arterial rise, the fluid rise lags slightly behind arterial and venous rise, due undoubtedly to the slowing of the adjustment between pressures inside and outside the skull, by straits in the system, particularly at the needle and at the aqueduct of Sylvius. There is no doubt in the mind of the writer that the venous rise is the result of the rise in arterial pressure, produced by the high arterial pressure exerted directly upon the intracranial structures, forcing the blood through the capillaries of the brain, thus raising the torcular pressure. Since the animal has been atropinized, there is no cardio-inhibition. The fluid rise is 60 per cent of the venous rise. We know from experiments cited earlier that a rise in venous pressure produces a rise in the fluid pressure, and that a rise in fluid pressure by itself produces no change in venous pressure, therefore it seems most probable that the rise in venous pressure is the cause of at least part of the rise in the fluid pressure. What has just been said regarding the rise in pressures applies equally well to the fall in the various

pressures, and it is thus obvious that a rise in the arterial and venous pressures produces a rise in fluid pressure, a fall in these pressures produces a fall in the fluid pressure.

The rise and fall in arterial pressure adequately explains all the changes in venous and fluid pressures because the rise and fall in all pressures are synchronous if we allow time for the venous and fluid pressures to adjust themselves in their complicated systems. There is evidence against rapid new formation and absorption of fluid with a synchronous rise in venous pressure due to increased fluid pressure under the influence of adrenalin. For while new formation might explain the rapid rise, and reabsorption the rapid fall, the latter assump-

TABLE 12  
*The effect of adrenalin after atropine on the various pressures*

TRACING	ARTERIAL			TORCULAR			FLUID		
	B	D	A	B	D	A	B	D	A
39	110	238	106	17	48	16	15	30	9
114	98	190	86	202	430	202	78	170	70
115	98	212	86	187	410	204	70	180	69
117	88	176	44	13	42	13	3	15	3
212	108	222	94	124	281	129	86	199	89
216	130	262	126	55	262	56	113	270	94
219	52	196	50	24	144	23	27	116	26
221	126	272	120	181	418	185	91	191	76
222	130	194	110	275	402	251	104	160	114
238	96	254	94	149	397	150	120	315	121
239	84	236	80	135	406	139	105	291	104
Average. >.....	101.8	222.9	90.5	123.8	294.5	124.3	73.8	176.0	70.4

tion is disproved by the fact that the readjustment is not delayed by ligation of the jugulars, nor is the curve following the administration of adrenalin materially changed by such a ligation.

We therefore conclude that the administration of adrenalin produces purely passive changes in the arterial, venous and fluid pressures, although rapid outflow of fluid follows such an injection if the outflow method of study is employed. (See fig. 13). Most recent writers hold this view regarding the effect of adrenalin on fluid formation, our only contribution is additional proof.

*e. Effect of raising arterial pressure, with the venous pressure remaining constant.* Having shown the effect on fluid pressure of raising arterial

pressure under conditions where there was a simultaneous rise in venous pressure, it is now necessary to show the effect on the fluid pressure of raising the arterial pressure independent of a rise in the venous pressure. Accepting the view that the only effect that adrenalin has on the fluid is a mechanical one—a point which we believe to be proved satisfactorily—we injected this drug intravenously to raise the arterial pressure. In order to maintain the venous pressure constant, the reservoir cannula in the torcula was attached on one hand to the manom-

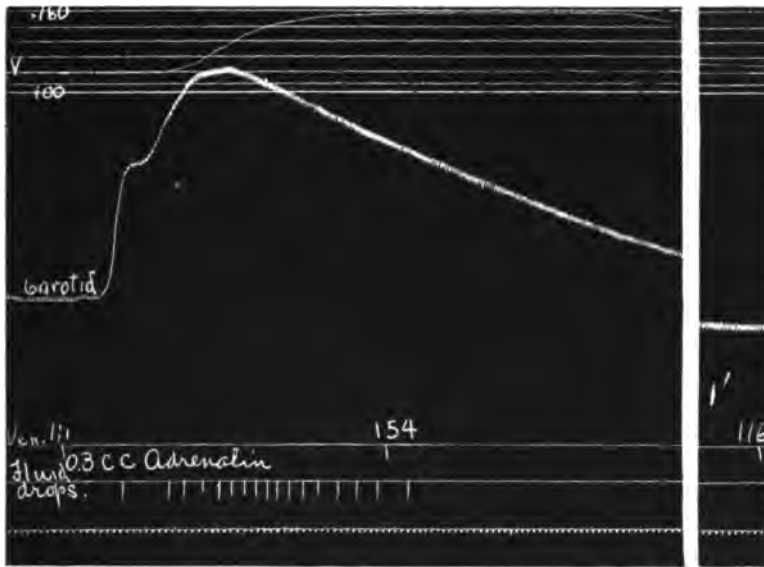


Fig. 13. This figure shows that if the outflow method is employed, the injection of adrenalin is followed by a marked rise in the torcular venous pressure,  $V$ , as well as in the arterial pressure, and a marked outflow of fluid. The outflow decreases on repeated injections.

eter, on the other to a short wide glass tube fastened in an upright position, and so adjusted that when the tube was full it exactly balanced the pressure in the sinus. Then when the venous pressure began to rise with the beginning of the adrenalin action, blood was forced over the end of the tube. In this way venous pressure was limited to that observed in the sinus at the beginning of the experiment. Some blood was lost by the animal during the experiment, but not enough materially to lower the blood pressure in the animal after the action

of the drug had ceased. Results from these experiments and a graph of one is seen in table 13 and in figure 14.

The results of the experiments recorded above, experiments carried out in four animals, show conclusively that if the venous pressure is maintained practically constant in the head by permitting the blood to escape from the sinus, and the arterial pressure is raised by the use of adrenalin, then the fluid pressure rises to almost the same degree as in the usual experiment, where the venous pressure rises with the arterial. From these experiments we conclude that arterial pressure,

TABLE 13

*Effect on fluid pressure of raising arterial pressure with venous pressure kept constant*

CONDITION	ARTERIAL			TORCULAR			CEREBROSPINAL FLUID			SUMMARY		
	B	D	A	B	D	A	B	D	A	Arterial rise	Venous rise	Fluid rise
Sinus closed	130	262	126	55	262	56	113	270	94	132	207	157
Sinus opened	126	254	108	59	69	59	97	226	71	128	10	129
Sinus closed	126	254	106	91	339	111	105	257	91	128	238	152
Sinus opened	124	250	110	73	95	57	95	206	85	126	22	111
Sinus closed	126	272	118	181	418	185	91	191	76	146	237	100
Sinus opened	122	270	114	175	205	180	82	218	83	148	32	136
Sinus closed	84	236	80	135	406	139	105	291	104	152	271	186
Sinus opened	78	226	94	129	129	129	102	279	101	148	0	177
Average sinus closed.....	116.5	256.0	107.5	115.5	356.2	122.7	103.5	252.2	91.2	139.5	238.2	148.7
Average sinus opened.....	112.5	250.0	106.5	109.0	124.5	106.2	94.0	232.2	85.0	137.5	16.0	138.2

if high, can and does influence the fluid pressure directly. A rise in the arterial pressure *can* produce a rise in the fluid pressure independent of a rise in the venous pressure. We would be more certain regarding these results if it were possible for us to take the venous pressure at some other point besides the point at which the fluid makes its escape, but to devise such a method seemed out of the question in the animals we were working with. An animal much larger than a dog would have to be employed. One observation made in some of these experiments is interesting: during the early pressor phase of adrenalin action the

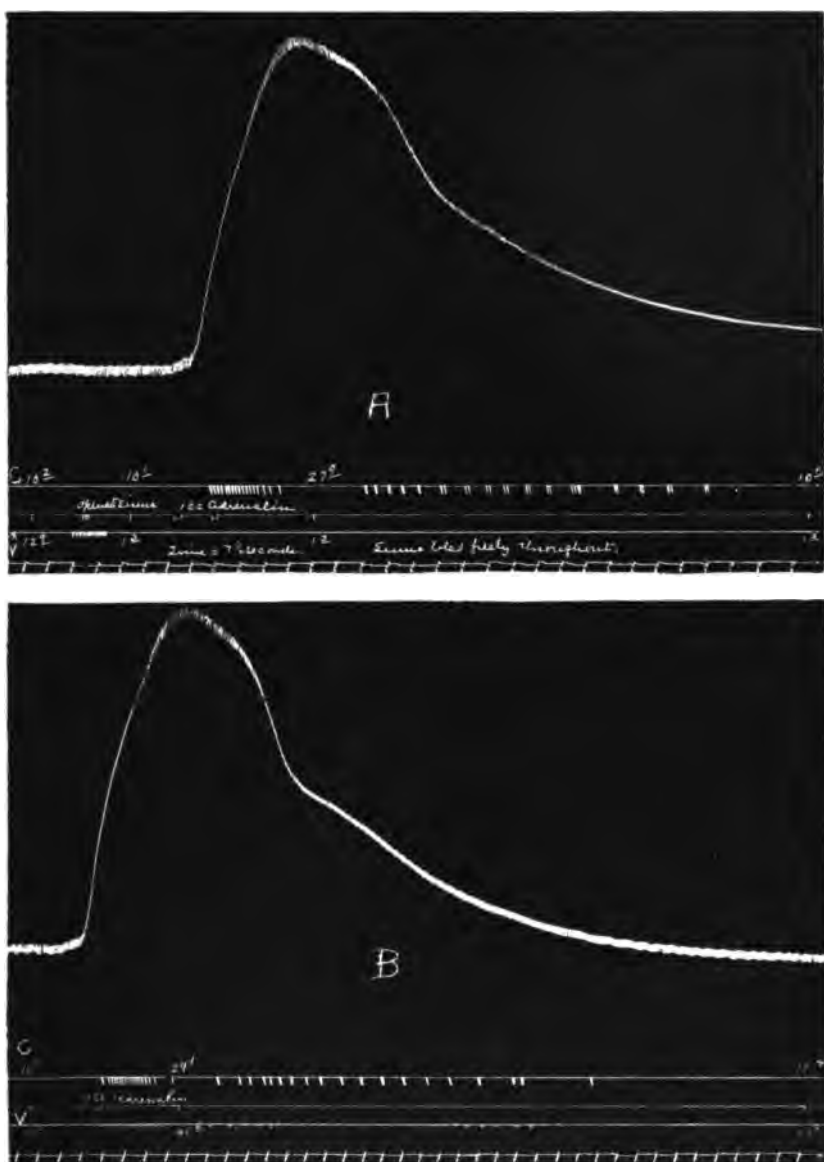


Fig. 14. This figure shows the marked similarity in the arterial and fluid curves no matter whether the sinus was open or closed. In *A*, the sinus was open and the fluid pressure rose from 101 to 279, the venous pressure remaining low on the injection of adrenalin. In *B*, the sinus was closed, the fluid pressure rose from 105 to 291, the venous rising from 135 to 406. For some reason the fluid pressure did not fall in *A* when the sinus was opened, as is usually the case (see fig. 5).

sinus bleeds freely, a considerable amount of blood flowing out over the end of the tube. Later, but before the pressure in the artery has reached its height, this outflow ceases. It is therefore evident that the source of blood for the sinus is cut off at the highest point in the blood pressure curve, probably by the obliteration of the capillaries by the high intracranial pressure. These experiments are contrary to the results reported by Howell. (See fig. 15, which proves that

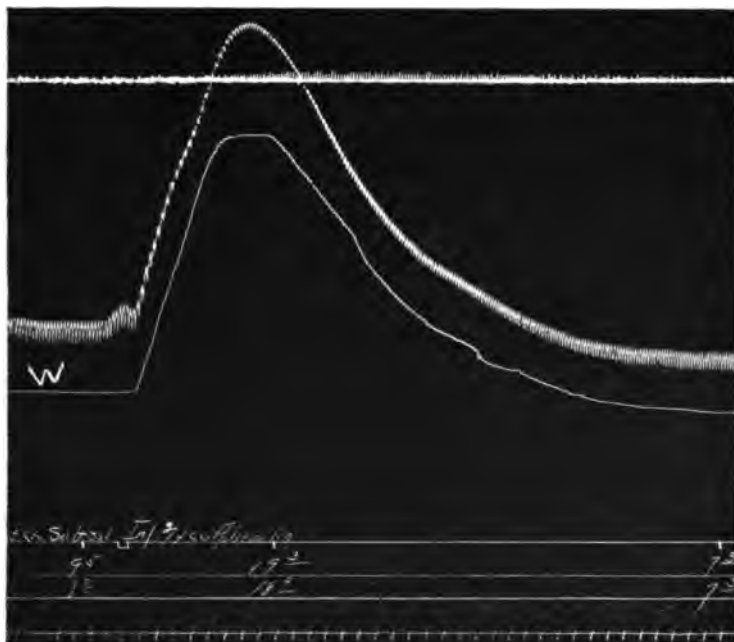


Fig. 15. This figure shows that in some instances the circle of Willis, W, is occluded during the action of adrenalin. This phenomenon was not frequently observed, in only about 2 per cent of cases.

parts of the circle of Willis are occluded during the height of the blood pressure rise with adrenalin).

From our experiments on the effects of venous pressure and of arterial pressure upon the fluid pressure it is evident that the pressure in the fluid is the result of the combined influence of at least two pressures—venous and arterial. Of these two the venous millimeter per millimeter is of much the greater importance, but under certain conditions the pressure of the fluid must be augmented by the arterial pressure. Under

these conditions the cerebrospinal fluid pressure is higher than the venous pressure in the torcula.

6. *The influence of the peripheral vagus.* The stimulation of the peripheral end of the vagus because of the profound effect it has upon the circulation and distribution of blood should have a marked effect upon the pressure of the cerebrospinal fluid.

Dixon and Halliburton (12) studied the effects of such stimulation and divided the results into three groups (p. 138):

(1) When the fall of blood pressure is small a slight passive diminution of the c-s pressure without any subsequent rise, even though the torcular pressure rises greatly; (2) an initial passive fall in pressure followed by a rise, which commences whilst arterial and venous pressures are still low; (3) an immediate rise in c-s pressure which may be regarded as being caused by increased secretion due to deficient oxygenation or the collection of carbon dioxide in the brain.

In table 14 we record the results from nineteen stimulations of the peripheral vagus on fourteen animals. We show in figure 16 what we consider typical results. So far as the table is concerned, the average result is a marked fall in arterial pressure early in the stimulation, with a gradual but very slow rise in the pressure, this is followed by a sudden rise when stimulation ceases followed by a gradual rise to normal. Numerous variations are seen. Such a marked fall in arterial pressure might in the skull either raise or lower the venous pressure although in the general circulation it commonly raises it. Our measurements show a fall in venous followed during the stimulation by a rise almost to normal, with a fall later below the level reached after the early effects of stimulation has passed off. The curve of the fluid pressure is exactly similar.

In figure 14, *A* and *B*, it will be noted that all the pressures fell simultaneously and rose simultaneously. In *A* the fall in all pressures was great, and the recovery was to above normal. In *B* the fall was much less marked and the recovery was incomplete in all the pressures. *C* is given to show that in some experiments there is a rather marked fall in arterial pressure but very little change in venous or fluid pressure. The decrease in cardiac output in these cases apparently is almost exactly balanced by the venous engorgement so that the venous pressure remains almost a constant. There is a rather large percentage of such cases seen in experimentation. We have never seen results such as reported by Dixon and Halliburton in figure 8, where torcular venous pressure rose markedly without raising the fluid pressure.

TABLE 14

*Effect of stimulation of peripheral vagus. This table shows the effect on arterial, venous and fluid pressure of stimulations of the peripheral vagus. On account of the nature of the response, two readings are recorded of the effect during and subsequent to the stimulation*

TRACING NUMBER	GENERAL ARTERIAL						TORSULAR VENOUS						CEREBROSPINAL FLUID					
	B		D		A		B	D		A		B	D		A		1	2
167	132	100	102	108	122	122	99	90	79	80	120	116	100	110	110	149		
175 <sup>a</sup>	122	112	108	140	126	126	210	210	209	209	202	118	118	120	120	108		
175 <sup>b</sup>	124	114	128	128	128	128	176	176	176	176	176	91	91	91	94	87		
183	144	128	128	154	154	154	69	70	80	78	70	115	105	100	115	115		
184	148	116	116	140	168	168	116	100	150	111	118	96	80	93	80	86		
185	166	116	140	154	164	164	109	100	117	116	114	85	85	86	85	83		
186	156	130	132	140	160	160	113	109	113	115	121	83	83	83	83	84		
187	164	140	162	162	158	158	121	121	123	123	125	86	86	91	91	91		
258	180	158	162	166	180	180	119	110	116	116	116	150	140	145	145	137		
259 <sup>a</sup>	180	160	162	176	180	180	143	140	131	130	102	137	137	134	130	90		
259 <sup>b</sup>	180	32	32	124	170	170	102	100	109	110	103	90	90	118	120	106		
260 <sup>a</sup>	180	170	174	184	182	182	110	111	164	160	124	101	110	155	150	102		
260 <sup>b</sup>	184	176	176	186	184	184	123	123	141	140	141	102	102	102	102	109		
260 <sup>c</sup>	188	164	170	190	186	186	141	140	101	101	109	99	99	99	98	104		
261 <sup>a</sup>	176	70	60	168	168	168	108	100	100	100	100	104	100	100	100	120		
261 <sup>b</sup>	124	30	30	146	154	154	75	70	90	120	106	77	80	100	110	99		
262 <sup>a</sup>	148	32	32	132	164	164	101	100	90	90	97	107	102	102	102	120		
263	150	126	128	142	142	142	169	160	145	145	136	191	190	158	160	162		
264	130	112	120	122	128	128	117	117	88	88	92	107	107	85	85	94		
Average	156.6	115.0	119.0	150.6	158.8	158.8	124.7	118.3	122.2	121.4	119.5	108.1	105.5	109.0	109.4	107.7		

1 = Records early change during and after stimulation.

2 = Records late change during and after stimulation.



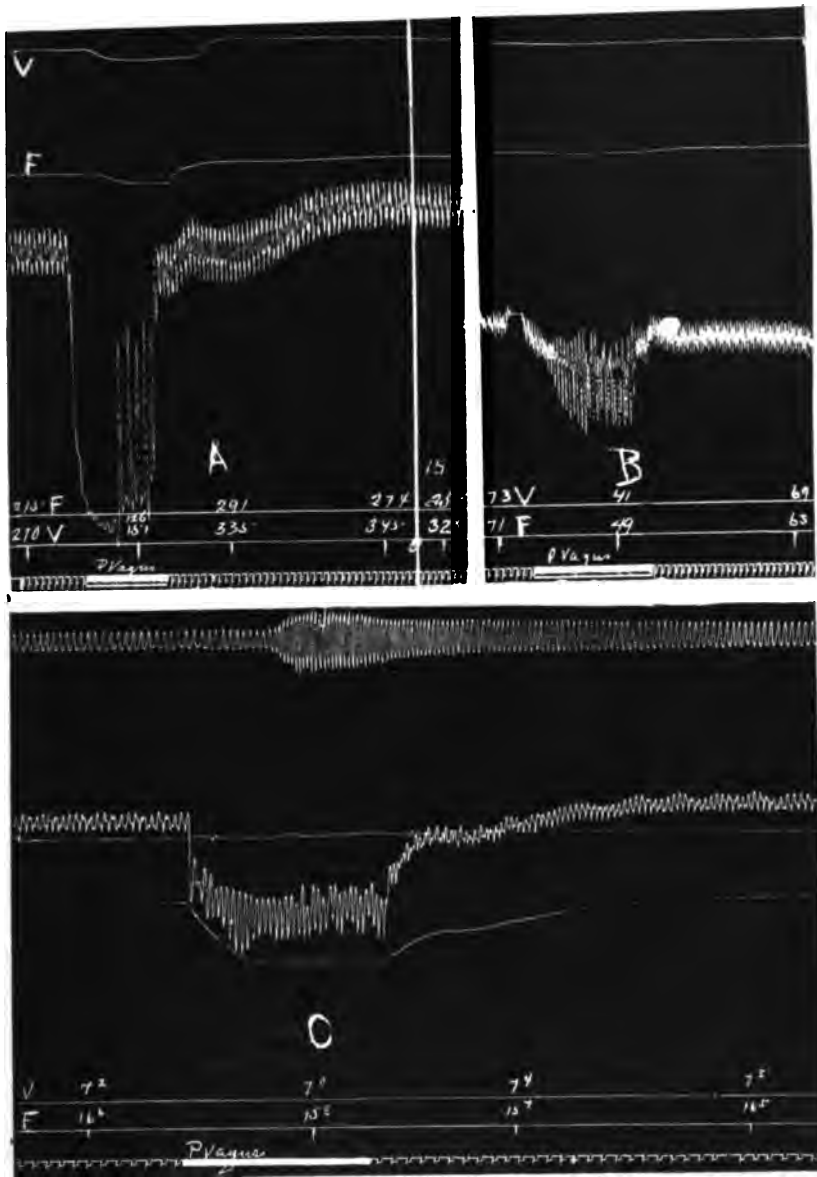


Fig. 16. This figure shows the effect on all the pressures of stimulation of the peripheral end of the cut vagus nerve. Note the marked differences in the changes of pressures in the different experiments.

Nor have we ever seen the result shown in figure 7, where arterial and venous pressure fell, but fluid pressure rose. We have no suggestions to offer as to the explanation of their results nor of our failure to observe similar ones.

So far as our experiments are concerned we are justified in the conclusion that the results of stimulation of the vagus upon the fluid pressure are exactly what are to be expected, if the changes are due to vascular changes in the skull. There is no evidence of new formation under the influence of the partial asphyxia produced.

7. *Influence of the amount of fluid in the canal.* One of the factors which influences the effect of a rise of the fluid or of an increase in the venous or arterial pressures is the amount of fluid in the canal. Early in the work we noticed that in rather a large percentage of animals the response of the fluid pressure to venous and arterial changes was small.

TABLE 15

*Dog, prepared as described above. Blood pressure raised by the intravenous injection of adrenalin*

	ARTERIAL RISE	TORCULAR VENTRICULAR RISE	CEREBROSPINAL FLUID RISE	FLUID RISE VENTRICULAR RISE
Normal.....	156	197	149	0.75
2 cc. fluid drawn.....	172	167	80	0.48
5 cc. salt injection.....	172	439	390	0.88
2 cc. fluid drawn.....	162	277	174	0.63

These records were marked "animal refractory to drugs" in our book. Later it was found that this refractory state could be changed to a non-refractory one by the introduction into the canal of a few cubic centimeters of warm salt solution. Similarly an animal which reacted well could be converted into a refractory one by the withdrawal of some of the fluid. The importance of this factor has already been brought out in the influence of the various bores of manometers on the rising and falling fluid pressure. In general the more fluid there is present in the canal the greater the response of the fluid to vascular changes. Many experiments were done to prove this point; there is some variation in the results but the statement made above holds, as may be seen from the following experiment (table 15):

As can be seen from this experiment the more fluid there is in the canal the more nearly does the rise of pressure in the fluid equal the rise in the pressure in the vein. The cause is so obvious as to need

no comment: It is an important factor in the response of the animal to the action of hemodynamic drugs, but one which has been neglected as is proved by the choice of method made by many of the workers in the field. The effect is a purely physical one. These experiments confirm also the statement made regarding the effect of the bore of the manometer tube upon the record of the fluid pressure, for it makes no difference whether the fluid is drawn out into a syringe or into a manometer tube, the effect on the pressure change is the same. We consider it highly probable that the amount of fluid varies within wide limits in different animals, hence the amount of response will vary widely, and many animals must be observed in order to get a reasonably accurate average. This fact explains why the fluid pressure was high even in those animals with an epidural hemorrhage in which case no blood entered the fluid, for it makes no difference whether the material is inside or outside the dura, so long as it lies within the bony calvarium, the result will be the same.

### 8. *Effects of respiration*

Among the physiological changes which *a priori* are likely to produce alterations in the fluid pressure or fluid outflow must be considered the effects of respiration, and in this connection not only must the effects produced by each individual respiration be considered, but also the effects of continued increased respiratory rate and depth. The oscillations produced in the fluid during each respiration are evident to anyone who will take the trouble to open the spinal canal of an animal under an anesthetic. As may be seen, in the ordinary case, with each inspiration the fluid falls, with each expiration, the fluid rises toward the hole in the wall of the canal.

*Literature.* The details of these changes have been carefully worked out by Knoll (26), and his observations can be fully substantiated. We do not believe that the criticism of Hill on this work is well taken, at least not for the experiments in which the loss of fluid from the canal is small. Previous to Knoll's work, Salathe (27) ascribed the pressure changes observed to take place during inspiration to the inspiratory rise in the blood pressure, while Haller and Fredricq (28) have ascribed the changes to alterations in the venous pressure occurring during the respiratory cycle. Knoll bases his conclusions that the pressure changes in the fluid are due to the changes in the venous pressure upon the following observations: *a*, The increase in the fluid pressure is not

exactly synchronous with the increase in the arterial pressure to the nervous system. *b*, The fluid pressure changes are modified but not lost by ligation of the arteries to the head, even when there was included the aorta close below the origin of the left subclavian artery. *c*, The pressure changes were synchronous with changes in the venous pressure, and in the same direction. *d*, One point against the venous origin of the pulsation in the fluid was the observation that ligation of the superior vena cava distal to the *vena azygos* and the inferior vena cava did not abolish the pulsation entirely. This he explained by the fact that in such a ligation the *vena azygos* is still open, and it is through this vein that the fluid pressure is still modified by changes in the venous pressure. This is rendered the more probable by the observation that in such conditions—ligation of the cavae—the small veins from the spinal plexus to the azygos system are markedly engorged with blood. He admits that in some cases the fluid may be so markedly influenced by the arterial pressure as to show an increased fluid pressure during inspiration and a decreased fluid pressure during expiration, a rhythm the reverse of the normal, but says that these cases are exceptional. This work of Knoll's has been carefully done, and is essentially correct, but he neglected to mention the influence on the pressure changes of alterations of the fluid volume in the canal,—this latter is an important one,—for unless there is a large volume of fluid in the canal, the arterial pressure can not change the fluid pressure directly and can modify it only indirectly by way of the venous pressure. The statements just made apply only to the changes occurring during a single respiratory cycle.

More recently Dixon and Halliburton (11) using the outflow method reached the conclusion that under artificial respiration "increased rate or larger thrust causes slower secretion, while decreased rate or smaller thrust, a faster secretion." They ascribe these changes to alterations in the  $\text{CO}_2$  content of the blood. To the same causes they attribute at least in part the increased outflow following chloroform inhalations in the normally breathing animal, an experiment in which according to their observations there is an increase in the outflow of the fluid.

*Experiments:* Our own work covers only a small part of the field: only the increased respiration due to stimulation of a sensory nerve, like the sciatic, and decreased respiration due to stimulation of an afferent inhibitory nerve like the superior laryngeal, or the central vagus, in the normally breathing animal. The effects of artificial respiration will be covered in a later paper.

*a. Increased respiration:* The effects of increased respiration upon the fluid pressure may be seen by referring to table 16. Experiments 500a to 501c were done on a dog in which stimulation of the sciatic always produced a fall in the blood pressure together with a fall in the venous and the fluid pressures. Experiments 504a to 505d were done on dogs in which stimulation of the sciatic always produced a rise in the arterial pressure, along with a rise in the venous and the fluid pressures. These statements hold true for both these animals not only for averages but for every individual experiment. The remaining five experiments were done on one other dog in which, as can be seen, stimulation of the sciatic always produced a rise in the arterial pressure, while the effects on the other pressures varied to some degree. The averages of the eighteen stimulations are shown in the final averages given at the bottom of the table. The conclusions to be drawn from these experiments are the following: The arterial, venous and fluid pressures rise in those cases where the stimulation of the sciatic has a pressor effect, and fall in those cases where that nerve has a depressor effect. The changes as shown by our eighteen experiments are all to be accounted for by alterations in the vascular pressure in the skull, for the pressure in the fluid varies in the direction to be expected from the alterations in arterial and venous pressures inside the skull. The averages of change between dogs, some of which show pressor, others depressor effects on stimulation of the sciatic, are well within the range of experimental error, and hence it is entirely unnecessary to assume either an increase or a decrease in the rate of formation of the cerebrospinal fluid. (See fig. 17).

Table 17 shows the results of increased respiration upon the outflow of fluid from a needle inserted into the 4th ventricle. The value to be placed upon this form of experiment has already been discussed in detail in our criticism of the methods of the study employed. However, we doubted some of the statements of recent workers regarding the behavior of the fluid on increasing the respiration. As can be seen from the table, in only two cases was there a decrease in the flow on increasing the respiration actively by sensory stimulation although it must be said that in many cases observed on other points in which the outflow method was used on increasing the respiration the fluid retreated out of sight into the tube, and did not reappear for some time. In ten cases recorded there was an increase in the outflow on increasing the respiratory movements, in two cases the rate was unchanged, and in three cases in this series the change could not be observed because of

TABLE 16

*The effects on the various pressures of increased respiration produced by stimulation of the sciatic nerve*

TRACING	VASCULAR AND FLUID PRESSURES												RESPIRATION			
	Arterial				Venous				C. S. Fluid				Rate		Amplitude	
	B.	D.	A.		B.	D.	A.		B.	D.	A.		B.	D.	A.	
500 { a b c	176	150	176		215	201	209		215	202	208		40	76	44	8
	176	156	174		209	199	207		208	197	207		48	76	40	8
	174	158	176		208	200	207		207	197	203		40	76	40	8
501 { a b c	168	154	176		201	198	206		201	194	208		33	60	34	7
	176	166	180		207	202	208		204	201	207		34	60	37	7
	180	160	176		208	201	207		208	199	206		37	68	37	7
Average.....	175.0	157.3	176.3		208.0	200.1	207.3		207.1	198.3	206.4		38.6	69.3	38.6	7.5
504 { a b c	150	176	160		87	100	99		140	140	144		62	70	64	10
	160	186	176		106	112	108		145	149	146		64	76	64	8
	170	190	180		111	111	101		149	141	132		64	84	64	7
505 { a b c d	174	196	184		111	115	109		140	146	131		64	96	64	6
	184	202	180		110	131	118		131	136	123		64	88	64	6
	160	204	188		118	141	120		123	137	125		64	92	72	6
	188	208	194		123	135	139		129	164	164		72	84	72	6
Average.....	169.4	194.5	180.2		109.4	120.7	113.4		136.7	144.7	137.8		64.8	84.2	66.2	7.0
300 { a b c d e	128	130	124		165	175	168		131	123	117		42	58	41	7
	130	144	128		168	177	152		118	115	109		44	59	43	8
	132	152	130		152	168	151		107	107	106		40	57	38	9
	130	140	130		157	169	149		106	110	104		46	58	44	8
	128	146	134		149	155	136		104	105	107		41	49	41	8
Average.....	129.6	142.4	129.2		158.2	168.8	151.2		113.2	112.0	108.6		42.6	56.2	41.4	8.0
Average of all expts.	158	164.7	161.9		158.5	163.2	157.3		152.3	151.6	150.9		48.6	69.9	48.7	7.3
																7.6

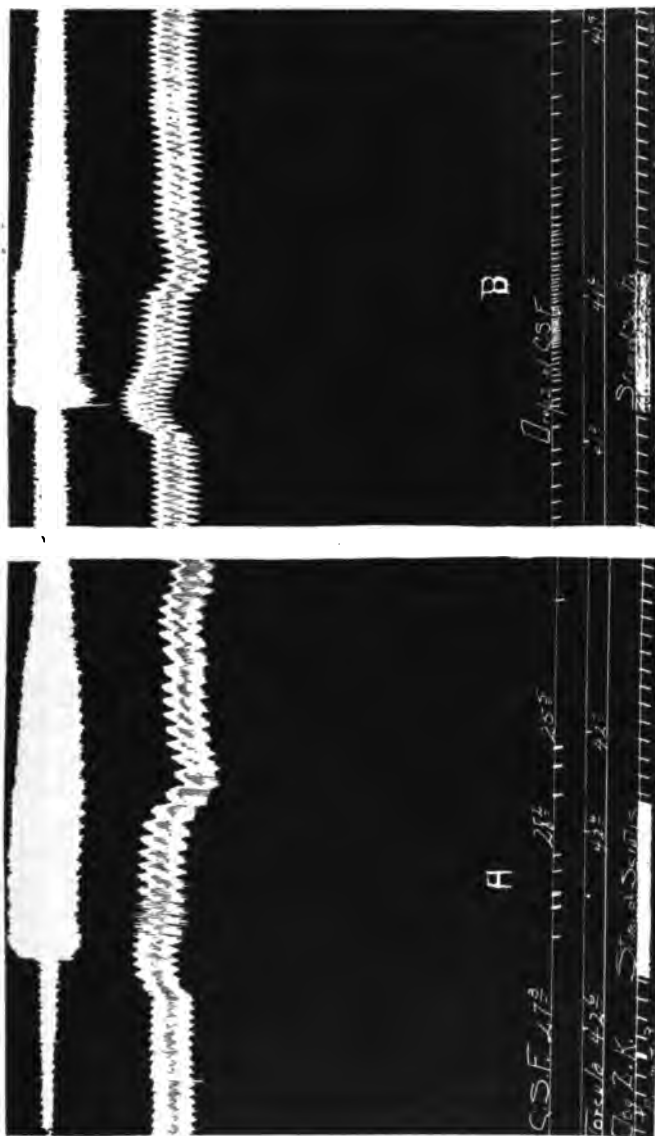


Fig. 17. This figure shows two experiments on the same animal: *A* is by the manometer, *B*, by the outflow method. Note that stimulation of the sciatic in *A* shows very little alteration in either fluid or venous pressure, while the same stimulation in *B* produces no marked change in venous pressure but a marked increase in the rate of outflow of fluid.

TABLE 17  
Effect on the arterial and venous pressures and on the fluid outflow of increasing the respiration by stimulation of the sciatic nerve

TRACING	VASCULAR PRESSURES AND FLUID RATE										RESPIRATION			
	Arterial				Venous			C. S. Fluid in drops per minute			Rate		Amplitude	
	B.	D.	A.	B.	D.	A.	B.	D.	A.	B.	D.	A.	B.	D.
502 <sup>a</sup> b	182 190	160 164	190 176	181 195	178 180	199 188	15.4 11.2	3.3 3.3	2.7 0.9	38 40	60 72	38 44	9 11	30 38
503 <sup>a</sup> b	174 184	130 180	182 180	169 171	155 155	167 179	12.0 11.9	2.1 1.5	1.9 2.0	33 32	64 68	33 40	7 11	46 43
257 b	194	202	192	412	426	419	8.0	35.0	10.1	38	45	39	6	16
256 <sup>a</sup> b	198 188	192 196	190 188	382 392	406 375	398 379	1.0 2.0	17.6 21.3	11.1 12.0	40 38	46 44	39 39	6 7	18 15
255 <sup>a</sup> b	190 190	194 194	190 188	299 261	319 363	342 359	20.0 2.0	29.2 13.3	15.0 6.0	48 46	58 48	46 46	5 10	12 16
254	198	214	194	419	426	420	29.0	40.0	20.0	44	54	46	3	14
253 <sup>a</sup> b	184 180	199 192	180 186	158 184	185 201	184 208	6.0 4.0	16.0 12.0	4.0 2.0	112 96	96 88	96 88	8 13	17 21
256 <sup>a</sup> b c d e	134 152 144 134 130	122 142 132 129 115	152 144 134 130 150	154 156 164 169 172	163 178 158 172 175	153 163 165 168 172	1.0 1.0 *0.0 *0.0 *0.0	1.0 1.0 0.0 0.0 0.0	1.0 0.0 0.0 0.0 0.0	56 60 64 70 76	84 90 80 80 84	60 64 70 76 75	3 6 7 6 3	55 64 32 25 30
Average.....	173.3	168.1	173.3	237.5	247.9	250.7	4.9	11.6	5.2	54.7	68.3	55.2	7.1	28.9
														7.7

\* No outflow. Fluid may have been drawn back into the tube.

† Decreased outflow on stimulation of the sciatic and increased respiration.

‡ Arterial and venous pressures decreased and fluid outflow increased.



limitations of the method employed. Taking an average of the seventeen experiments, the rate of outflow was more than doubled by increasing the respiratory movements. Therefore, the conclusion is warranted that on increasing the respiration by stimulation of the sciatic in a normally breathing dog the outflow of fluid may be increased or decreased, not always decreased as is the statement of Dixon and Halliburton (11) for their experiments where artificial respiration was increased. Their figure 13 (p. 237) as shown in their article does not substantiate their claim that augmented secretion commenced before the respiration was visibly affected, for if the time relations of their recording apparatus are correct, the increased outflow *did* come synchronously with the decrease in amplitude in the respiratory movement, and the maximum was reached at a time when the respiratory movements were approaching the minimum. There was little or no change in the rate of respiration so far as can be judged from their tracing until late during the inhalation and then there was an increase. It has been our experience that by no means does every decrease in the respiratory movements result in an increase in the outflow, nor does every increase in the respiratory movements result in a decrease in the outflow of the fluid. Further, these experiments are inconclusive because they do not measure—at least do not record in their paper—the alterations in the arterial and venous pressures during the experiments involving a decrease in the respiratory movements. Neither do they give the results from more than one experiment, either by this or by the pressure method, an omission sufficient to make their conclusions of little value. It will be noted that in both those cases where there was decreased outflow there was a fall in both the arterial and venous pressures, which may have been the cause of the decreased outflow. On the other hand examination of the table shows that in some of the other experiments where there was a similar fall in both these pressures there was an increase in the outflow. Hence a fall in both venous and arterial pressure is not invariably followed by a decrease in the outflow of fluid, and we believe that the cause of the increased outflow is due in some cases to increase in the arterial or venous pressure or both, accompanied by an increased accumulation of blood in both arterial and venous channels, and in some cases to an increase in the oscillation of the contents of the canal, permitting the outflow of more fluid through the needle, just as more fluid will flow out of a hole in the side of a tank if the fluid in the tank is agitated more strongly. Another explanation is that some of the straits in the intra-

dural canal are always more or less completely occluded by the structures passing through them, thus hindering the free passage of fluid from one portion to the other. Thus if the cranial fossae were full of fluid, increased respiratory movement would, by the rise and fall of the mid-brain during dyspnea facilitate the passage of fluid from the skull cavity to the medullary region and thus increase the outflow from a needle inserted in that region. On the other hand if the cranial fossa were nearly empty, then increased respiratory movements would make easier the passage of fluid from the region of the medulla into the cranial cavity, and thus decrease the outflow. We believe this, together with the alterations in arterial and venous pressures, to be the explanation of the changes observed, these changes being in the nature of either an increase or decrease when the respiration is stimulated by the action of the sciatic. Changes due to alteration of rate and depth of artificial respiration have not been studied, but we do not believe that they will differ from the changes already described. However, they are to be submitted to later study. Thus the decreased flow is due to fall in arterial or venous pressure or both, with less blood in the vessels thus allowing more space for the fluid, and reducing the outflow, or in many cases the fluid may—under the influence of strong respiration—be distributed to distant parts of the canal thus reducing the outflow through the needle. The increase is due to the increases in arterial and venous pressure which displaces more of the fluid, or to the accumulation of fluid in the medullary region by the release of fluid from other parts of the system by movements of the central nervous system.

*b. Decreased respiration.* Having studied the effects of the increased respiratory movements, we undertook the study of the effect of decreased respiratory movements as produced by stimulation of the superior laryngeal and central vagus. Table 18 shows the effect of stimulation of the superior laryngeal upon the pressure of the fluid. The table shows the results of twelve stimulations performed on three dogs. As can be seen from the tables, the averages of all the pressures rise and then return to the normal immediately after the cessation of the stimulus. The fluid rises relatively more in these animals than for most of the animals shown in this work but in all cases high normal pressures were existent at the beginning of the experiment; thus the canal was full of fluid and the fluid pressure was influenced by arterial as well as venous. If the change in the fluid pressure had been one of secretion then the fluid pressure should have remained high for some

TABLE 18  
*Effect of the stimulation of the superior laryngeal on the arterial, venous and fluid pressures*

TRACING	CIRCULATION AND FLUID								RESPIRATION					DURATION OF STIMULUS seconds						
	Arterial				Venous				Fluid			Rate			Amplitude					
	B.	D.		A.	B.	D.		A.	B.	D.	A.	B.	D.		A.	B.	A.			
306	108	102	106		238	257	262		222	233	228		24	9	24	3	1	3	41	
{ 307	118	120	116		341	349	340		334	346	337		52	33	44	3	1	3	12	
	116	118	110		329	326	311		328	327	311		44	36	40	3	1	3	22	
	108	116	112		306	249	281		308	297	280		40	39	52	3	1	3	38	
		108	116	116		279	336	333		277	326	316		50	0	56	3	0	3	22
{ 310	168	190	164		130	146	135		189	213	211		32	0	32	15	0	15	15	
	144	136	140		116	119	122		205	206	218		44	0	44	12	0	10	26	
	142	135	146		127	130	126		228	230	235		52	0	49	10	0	10	20	
		144	138	142		126	132	130		236	237	236		26	20	23	10	2	10	22
{ 111	142	138	140		131	135	131		231	238	235		23	23	20	23	10	3	10	22
	140	138	140		132	140	134		236	239	238		23	20	24	10	3	10	47	
	140	138	140		133	140	132		238	241	239		24	21	24	10	7	10	45	
		140	138	140		133	140	132		238	241	239		24	21	24	10	7	10	45
Average...	131.5	132.0	131.0		199.0	204.9	203.0		252.6	261.0	257.0		36.1	16.5	36.2	7.6	1.5	7.5		

time after the stimulation ceased. Such was not the case; in fact, as can be seen from the table, it fell to almost exactly the same proportional amount as the venous pressure. The venous pressure rose 4.3 mm. and fell 1.9 mm., hence the fall was equal to 44 per cent of the rise. The fluid pressure rose 8.4 mm.—due undoubtedly to changes in the arterial pressure for the fluid pressure was high—and fell 4 mm.

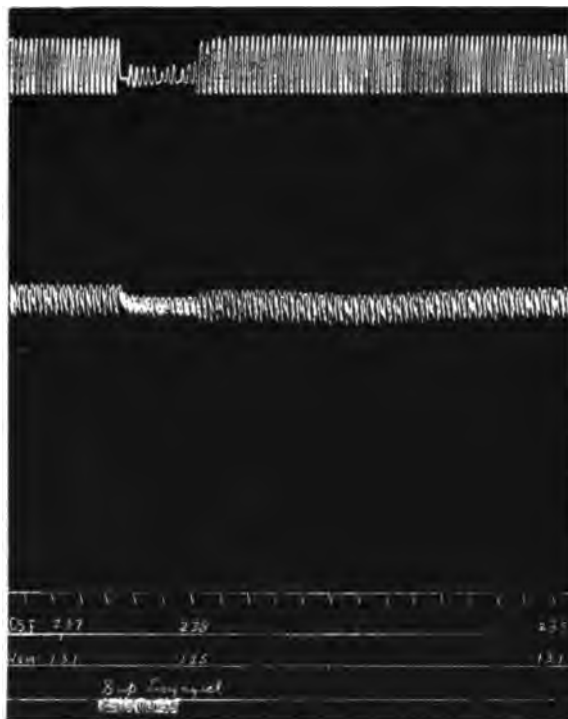


Fig. 18. This figure shows the effect on all the pressures and upon respiration of stimulation of the superior laryngeal nerve.

or 44 per cent of the rise. If the observations had been prolonged until the venous pressure was normal, then the fluid pressure would also have been normal. The period of stimulation varied from 12 to 47 seconds—as long as it is possible to inhibit respiration by such stimulation. (See fig. 18).

Inhibition of respiration by stimulation of the central vagus was carried out. Table 19 shows the results from eight experiments on two

TABLE 19  
*Effect of the stimulation of the central vagus on the arterial, venous and fluid pressures*

TRACING	CIRCULATION AND FLUID										DURA- TION	RESPIRATION					
	Arterial			Venous			Fluid					Rate			Amplitude		
	B.	D.	A.	B.	D.	A.	B.	D.	A.	B.		D.	A.	B.	D.	A.	
312	140	186	140	130	180	136	234	300	238	64	38	46	34	10	7	9	
313	140	154	140	138	198	156	236	315	255	158	36	0	17	7	0	9	
308 { a b c	190	204	188	149	181	153	158	169	156	38	68	0	76	14	0	14	
	192	200	196	142	161	155	144	153	139	45	76	0	68	14	0	14	
	190	210	200	144	179	154	139	151	131	45	68	0	68	14	0	14	
308 { a b c	138	142	124	317	319	295	294	297	266	22	20	0	20	2	0	2	
	124	120	114	289	285	271	261	264	246	77	20	5	28	2	1	2	
	112	114	108	259	261	259	239	242	231	45	28	20	24	2	1	2	
Average.....	153.2	166.2	151.2	196.0	220.5	197.3	213.1	236.3	207.7		44.2	8.8	41.8	8.1	1.1	8.2	

animals. The inhibition was maintained from 45 to 158 seconds, ample time—according to Dixon and Halliburton—for asphyxiation or  $\text{CO}_2$  to have its effect upon the mechanism forming the fluid. The changes, as might be expected, are much greater than those secured from stimulation of the superior laryngeal, because of the greater number or the greater effectiveness of the afferent fibers in the vagus trunk. The arterial, venous and fluid pressures all rose with the beginning and fell with the termination of the stimulation. The changes in all the fluids are rapid, synchronous, in the same direction, and to some degree proportional. Therefore, the changes are all due directly or indirectly to the effect of the pressor fibers, and the changes are purely mechanical in origin, coming from changes in the blood pressure. Much work remains to be done on this phase of the subject, but the data we have given are ample to prove that the conclusions of Dixon and Halliburton regarding the influence of  $\text{CO}_2$  in stimulating the structures forming the fluid are too sweeping, being based upon the results from experiments too few in number and not sufficiently controlled to warrant the broad conclusions they have drawn. We have experiments exactly like those shown by these authors. The conclusions drawn from their graphs would be correct if the graphs were typical of a larger number of experiments, but they are not, as can be seen by reference to our tables given above. Whether the outflow method they employed or the manometer method is correct has already been discussed (see fig. 19).

*Asphyxia.* The effectiveness of asphyxia as a stimulant of the mechanism forming the cerebrospinal fluid has recently been emphasized. Dixon and Halliburton using the outflow method state: "asphyxia always leads to considerable increase in outflow." This they believe due to the  $\text{CO}_2$  of the alveolar air, and point out that in this respect the choroid does not behave like a secretory gland, for most glands secrete more rapidly when the blood is well aerated. They state that drugs such as nitrites and nitrobenzenes which rapidly produce methemoglobin also cause the secretion to increase. In their later article dealing with fluid pressure they claim that torcular pressure rises slowly at first during asphyxia, and then more rapidly, and continues to rise when the arterial pressure falls and the heart fails. The cerebrospinal fluid pressure rises slowly at first, but more rapidly later. The fluid pressure commences to fall before the arterial blood pressure has reached its maximum (fig. 20).

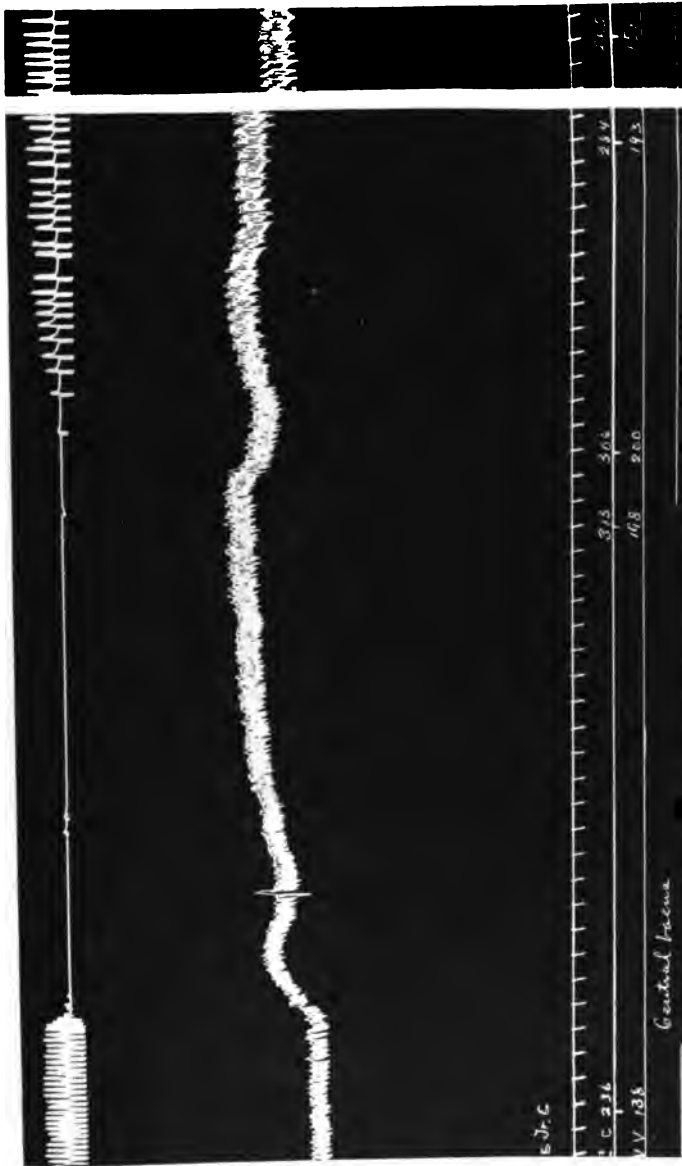


Fig. 19. This figure shows the effect on all the pressures of stimulation of the central end of the cut vagus nerve.

We show in table 20 the beginning, maximum and end pressures in eight animals well anesthetized and killed by asphyxia produced by opening one or both pleural cavities by large incisions parallel with and between the ribs. We preferred this method to deep anesthetization, because with the interruption of artificial respiration the latter method will produce too marked a depression of the centers of the medulla. Using this method of study we found that torcular venous pressure rose with the asphyxial rise in arterial pressure, and this rise of venous pressure persisted after arterial pressure had fallen to zero.

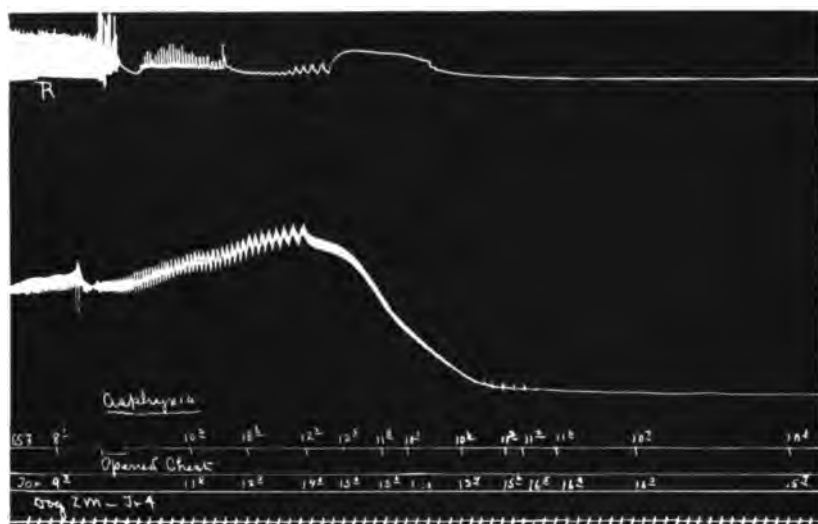


Fig. 20. This figure shows the effect of opening the chest (asphyxia) upon the respiration, arterial, venous and fluid pressures. The animal was under moderate surgical anesthesia.

It was our experience that fluid pressure ran a course parallel with the venous pressure. Venous pressure rose on the average 67.9 mm. and fell 44.5 mm. Fluid pressure rose on the average 71.0 mm. and fell 53.5 mm. The venous pressure fall was 65 per cent of the venous rise, and fluid fall was 75 per cent of the fluid rise. In other words, these two pressures run a remarkably parallel course, both remaining above the normal. Where the outflow method is used this might in many cases result in increased outflow of fluid. In figure 20 we show a typical result. Note that venous and fluid pressures run a remarkably parallel



course. There are two maxima in the curves, and while not absolutely synchronous they fall closely enough together for the phenomenon to be significant. The first maximum in fluid pressure undoubtedly came early because of the marked rise in arterial pressure, for arterial pressure does influence fluid pressures in some animals, as has already been shown.

In accordance with the findings shown in table 20 and figure 20, we believe that all the changes observed in fluid pressure during asphyxia are logically explained by the changes in arterial and venous pressures. Thus it is unnecessary to assume new formation under the influence of carbon dioxide.

TABLE 20

*Asphyxia. This table records the variations in arterial, venous and fluid pressures between the opening of the chest and the death of the animal. Note that the pressures all rose and then fell, but the fall in arterial was much greater than the fall in venous and fluid pressures*

ARTERIAL			TORCULAR VENOUS			CEREBROSPINAL FLUID		
B	D	A	B	D	A	B	D	A
120	144	30	92	156	157	81	188	106
80	126	42	65	170	84	24	160	38
120	152	124	102	136	134	104	142	141
64	132	128	68	145	110	58	133	92
94	118	22	205	240	165	87	140	82
80	84	44	79	119	56	87	130	56
112	180	16	38	232	131	25	142	102
80	86	8	116	110	115	113	112	102
Average 93.7	127.7	59.1	95.6	163.5	119.0	72.3	143.3	89.8

#### IV. THE NORMAL RATE OF THE FORMATION OF THE CEREBROSPINAL FLUID AND ITS ABSORPTION

##### *Formation*

Logically, the first question to settle in this problem is the normal rate of the formation of the cerebrospinal fluid. Various estimates have been given. Dixon and Halliburton (11) record the rate in the dog as averaging about 6 cc. per hour in an experiment lasting almost three hours; in the goat as varying between 13.5 cc. during the first hour to nothing after the fifth hour. The decrease was gradual. We assume that these figures do not include the fluid which escaped when

the membranes were first punctured. Frazier and Peet (7) obtained from a dog of 10 kilo weight 1.66 cc. in 7 minutes and 10 seconds, or 0.231 cc. per minute; from a dog of 8 kilo weight they obtained 3.57 cc. in 18 minutes, or 0.192 cc. per minute. They had drained the canal 10 minutes before the observations were begun. In these experiments, also, the rate decreased during the experiment.

Recalling the errors of method pointed out above, it is easy to see that this so-called "normal rate" of flow recorded by these authors may have no relation whatever to the normal rate of formation of fluid. This flow may represent the slow *leakage* past obstructions in the canal of fluid not removed by the preliminary drainage, a leakage made possible by the rhythmical movements of the cord and medulla, produced by the respiratory and cardiac impulses. Since these movements are uniform the rate of leakage is uniform but gradually decreasing as the reserve is exhausted, and finally ceasing entirely. This interpretation of the flow easily explains the observation of Dixon and Halliburton that the flow in the goat ceased entirely after the fifth hour although the blood pressure was still 80 mm. of mercury, and they had "frequently noted abundant secretion at a much lower pressure." On the other hand, the flow may represent *transudation*, not the normal formation, transudation from the meninges or other vascular structures of the dural canal as a result of the decreased intracranial pressure produced by the more or less complete drainage fluid. Frazier and Peet point out the importance of this factor in commenting on the clinical observations in compound fracture of the skull, but failed to apply their criticism in the selection of a method for their own experiments. Of course if the criticism is valid in the clinical cases it is valid in the laboratory experiment. Of the two factors, it seems probable the first—leakage of preformed fluid—is more important in experiments of short duration like those cited above; while the second—transudation from reduced intracranial pressure—is more important in experiments of long duration, into which class the clinical cases fall.

In the course of our work we had ample opportunity to study the "rate of formation of the fluid" according to the methods of the authors mentioned. Several experiments were done to determine this point specifically. However, after considering the situation fully we decided that the data did not bear upon the point at all. We could and did measure the rate at which the fluid would drop from a needle inserted into the canal, or would run out into a glass tube connected with the

needle, but that in our opinion does not measure the rate of formation of the fluid. Neither the tube nor the manometer method, nor any other method we have seen described is suited to make this apparently simple determination. All that has been measured is the rate at which fluid can be moved about in the canal, or the rate at which it can be formed under reduced pressure within the skull. If an animal is observed over a long period of time, only slight variations of the level of fluid occur, so long as the level of venous pressure remains unchanged and the respiration is constant. If the anesthetic is reduced, so the venous blood pressure rises and the respiration increases, then the fluid rises in the tube. When the anesthetic is increased, and venous blood pressure and respiration decrease, the fluid falls in the tube. In experiments lasting from three to five and one-half hours the change of fluid levels are negligible so long as the venous blood pressures remain the same. The explanation of the constancy of the fluid levels is easy. Normal conditions within the skull are retained and hence formation and removal of fluid take place by the normal mechanism, and at the normal rate, and therefore balance exactly. Only when vascular changes take place rapidly does the level of fluid vary to any great degree. These changes of course are measured readily, and their influence upon the level of the pressure of the cerebrospinal fluid can be estimated.

When the manometer method is used the same results are obtained; there are no changes which can not be explained by the change in blood pressure.

Without being able to give direct and convincing evidence on this point on account of the lack of a suitable method, we believe that the normal rate of formation of the fluid is a relatively slow process, much slower than the statements of the clinicians and the experiments cited above from other workers would lead one to believe. We do not wish—as the above statement seems to do—to put this problem out of the realm of experiment, but it seems to occupy that place because of the lack of a suitable method.

Attempts to get at this problem indirectly have led to questionable and we believe erroneous conclusions. Frazier and Peet, working with phenolsulphonaphthalein found that if the drug is injected into the ventricles “under normal conditions about 50 to 60 per cent is excreted into the bladder within two hours.” They then conclude: “If we can assume that the cerebrospinal fluid is absorbed proportionally as rapidly as the amount of phthalein injected, *and there is no reason*

*for believing otherwise*, we are led to the conclusion that at least 50 to 60 per cent of the cerebrospinal fluid is absorbed every two hours." We cannot agree with this conclusion. The absence of proof that an assertion is untrue does not prove that the assertion is true. In this instance it cannot be assumed, it must be proved, that all of the constituents of the cerebrospinal fluid are absorbed in the same proportion to the whole as the drug; otherwise the possibility of the selective absorption of a foreign material is not eliminated. It would be just as sound reasoning to assume that since from 50 to 60 per cent of the phenolsulphonephthalein has been excreted from the blood by the kidneys in two hours that 50 to 60 per cent of the water and other constituents of the blood had been eliminated in that time. Assuming the weight of the blood to be one-tenth of that of the body for animals, and the blood to be two-thirds plasma, than in a man of 60 kilos weight there should occur in 2 hours the excretion of 2 to 2.4 liters of urine, or 24 to 28.8 liters in 24 hours. This of course is preposterous and proves that the drug must be selectively excreted from the kidney and is not excreted in proportion to all the other constituents of the blood. This same activity must be proved to be absent from the cerebrospinal mechanism before the conclusions of Frazier and Peet become at all convincing.

Dandy and Blackfan (6) made observations on the same field at about the same time. Their results are in accord with those of Frazier and Peet but from their findings they drew no conclusions regarding the rate of removal and hence of formation of the cerebrospinal fluid.

We do not claim to have proved a specific absorption of pilocarpine from the dural canal but using the submaxillary gland as an index of the presence of the drug in the blood, we did establish the fact that absorption of this drug from the canal is approximately the same in rate as from other internal cavities of the body. (See tables 21 and 22).

From table 21 it becomes evident that absorption from the dural canal of pilocarpine, or rather its transfer into the blood stream, is accomplished after about the same latent period as the transfer from the bowel, pleural or peritoneal surfaces. Since the transfer from these surfaces is in all probability by specific absorption of the drug *per se*, it follows that so far as the time element is concerned there is nothing against the transfer of the drug from the canal to the blood by the same process. As was stated above we are fully aware of the fact that this does not *prove* that drugs are specifically absorbed from the canal. In this connection it was considered important also to study

TABLE 21

*Rate of absorption of pilocarpine. Wharton's duct was used unless otherwise indicated*

DOG	MODE OF APPLICATION	LEFT DUCT	RIGHT DUCT	REMARKS
		minutes	minutes	
1a	Intradurally	4.83		
2a	Intradurally	4.09		
3a	Intradurally	3.68	3.78	Right chorda cut
1	Intramuscularly	0.78	0.87	
2	Intramuscularly	1.00	1.04	
3	Intraperitoneally	3.00	3.20	
5	Intraperitoneally	2.7	2.9	
6	Intraenterally	2.05	2.9	
9	Intraenterally	11.0	12.0	
7	Intrapleurally	5.2	3.6	
8	Intrapleurally	4.2	3.8	
10	Subcutaneously	2.15	2.13	
11	Subcutaneously	4.51	3.11	Left duct twisted
12	Subcutaneously	4.45		
13	Subcutaneously	3.4	3.2	
15	Intraocularly		4.25	
16	Intraocularly		3.08	

TABLE 22

*Latent period of the absorption of atropine from the dural canal as judged by the effect of the drug on the submaxillary gland and the heart. The circulation and the gland had previously been influenced by the injection of pilocarpine*

TRACING	GLAND	HEART
	minutes	minutes
1	1.0	2.18
2	6.6	*
3	2.58	*
4	1.55	0.50
5	1.86	*
6	0.75	1.25
7	4.35	*
8	1.63	*
9	4.33	*
10	0.43	0.41
11	0.33	0.33
Averages.....	2.31	0.93

\* Beginning of action not clearly marked.

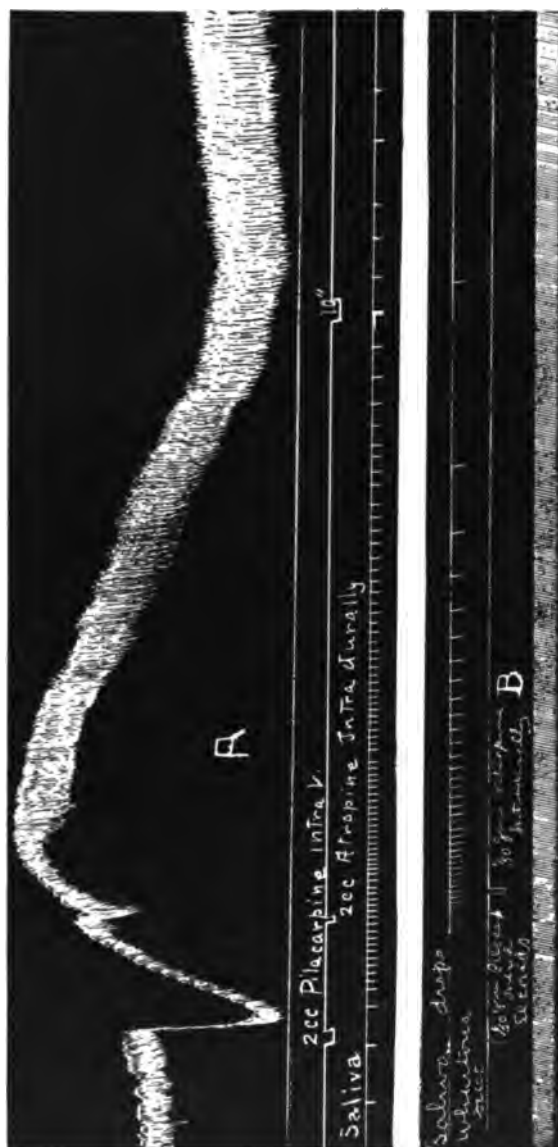


Fig. 21. This figure shows the rate of absorption of pilocarpine and atropine from the dural canal. Note that the latent period is about twice as long when the drug is given intradurally as when given intravenously. The rate of speed is about the same in both A and B.

latent periods of absorption of atropine from the dural canal. As may be seen from the table, there is a wide variation in the duration of this period independent of any known cause, for the injections were always made in exactly the same way, and in equal amounts in all the animals. A cannula was placed in Wharton's duct and the chorda lingual was stimulated electrically until saliva dropped from the end of the tube. The animal was then injected subcutaneously with pilocarpine, and the cardio-inhibitor and salivary mechanism thrown into activity. Then atropine was injected intradurally and the release of the heart from inhibition and the cessation of salivary flow observed. As is well known it is not always possible to judge the exact moment of release of the heart from the inhibition mechanism; for that reason but few observations were made on that point. The results of eleven experiments are shown in table 22 and in figure 21.

Since obvious error in the direct experiments can be pointed out, since the conclusions regarding the rate of formation based upon the rate of excretion of phenolsulphonephthalein can be shown to be unwarranted, it becomes evident that the normal rate of formation of the fluid has not been determined.

#### *Absorption of the fluid and its contents*

*Literature:* It has long been known that substances like dyes injected into the subarachnoid space, find their way into the blood. From experimental evidence it seems that the absorption is by way of the blood stream rather than by the lymphatics. Further, there is evidence for believing that no direct communication between the subarachnoid space and the blood vessels exists, hence the transfer from this space to the blood is by filtration, by diffusion or by direct and specific absorption. Since the hydrostatic pressure is usually—some say always—higher in the blood vessels than in the fluid, it is evident that filtration cannot be the mechanism of transfer, hence diffusion and specific absorption are left as the only alternatives. Dixon and Halliburton (29) in a recent article claim to have established the fact that substances which disappear from the fluid do so by a process of diffusion. They assume that a molecule of adrenalin is smaller than a molecule of secretin, and that a molecule of secretin is smaller than a molecule of pepsin. Then they claim to have shown that the rate of transfer of these substances to the blood is slower or faster in proportion to the size of the molecule of the substance they are studying. That their work does

not prove the point will become evident. The point at which the transfer from the canal to the blood takes place is still a matter of controversy.

The rate at which this transfer takes place has been studied for years. Much of this work is of no value from the standpoint of a study of the normal, because of the abnormal pressures used in producing the results. The present communication deals with the absorption of drugs at or near normal pressure conditions.

Lewandowsky (30) studied the absorption of strychnine and other substances from the dural canal, and concluded that these drugs entered the nervous system by direct absorption from the cerebrospinal fluid, because he could produce typical results with smaller doses by this method than by the ordinary route—by subcutaneous injection.

A further study (31) showed that it was possible, by intradural injection of strychnine, to produce local areas of hyperexcitability in the cord without influencing remote areas. This also points to direct absorption of the drug by the nervous tissue from the fluid instead of from the blood, for in the latter case all parts of the nervous system would be simultaneously and proportionately affected.

Meltzer and Auer (32) studied the effect of the intradural injection of adrenalin in six monkeys, used repeatedly, with several days intervening between experiments. Twenty-one injections were made in all. They concluded that intradural injections do *not* produce the typical effects of an intravenous injection. Thirteen of these injections gave a rise in blood pressure, slow in making its appearance and of long duration. In six cases a fall preceded the rise, and in one case the fall was the only effect. In seven cases there was a rise similar to that seen on intravenous injection. They suggest that this rise is due to some of the solution entering a vein at the time of the injection. Recently (33) these same writers have restated these same facts with additional proof.

Dixon and Halliburton in their last paper on the fluid state: "after an injection (of adrenalin) into the subcerebellar cisterna, a short latent period ensues of from ten to thirty seconds, then cardiac acceleration becomes obvious (the vagi being cut) and the blood pressure rises." Regarding nicotine they say: "Nicotine in doses of from one to two milligrams acted like adrenalin on the vascular system, and was readily absorbed from the cerebrospinal fluid into the blood." In support of this statement they give a tracing of the action of each drug which shows the blood pressure curves of intravenous and intradural injection almost superimposed. From these findings supplemented by observations



upon substances with a supposedly larger molecule like secretin and peptone, and upon crystalline substances and dyes, they draw the following conclusions: "If these substances (introduced into the fluid) are readily diffusible the speed with which they appear in the blood is very remarkable. . . . An injection of substances like adrenalin, nicotine and atropine produces characteristic physiological effects almost as rapidly as if injected in the venous circulation. Substances which are not diffusible such as proteins . . . do not produce their characteristic effects, when introduced into the cerebrospinal fluid. Whereas substances of intermediate molecular size, such as secretin, diffuse slowly, and cause their action at an intermediate rate." Numerous other conclusions were drawn. The conclusions regarding the action of adrenalin and nicotine as drawn by Dixon and Halliburton are warranted from the tracings they publish. But the writer is convinced that the facts regarding the action of these two drugs are not as stated by the authors quoted.

*Experiments:* In experiments carried out on twenty-one animals into which forty separate series of injections were made, twenty-seven of which were with adrenalin, thirteen with nicotine, in only two instances did the arterial pressure rise in the manner described by Dixon and Halliburton as the usual result of the injection. In both cases this rise followed the injection of adrenalin, and in both cases the cerebrospinal fluid was bloody when fluid was withdrawn as a preliminary step to the injection of the drug. In one of these cases a later injection of adrenalin was followed by a fall instead of the rise described as characteristic by the authors cited. We are forced to the conclusion that some error was made by the authors probably in the injection of the drug which resulted as suggested by Meltzer in his own work, in the entrance of a part of the solution into the blood stream, probably in this case by way of some of the intracranial veins, with the production of a characteristic rise in the blood pressure. We noted the results given in two cases and were able to produce results in two others like those reported by them as the normal, but in the four cases in which this rise was noted the fluid was bloody in two, and a communication existed between the subarachnoid space and the torcula in two. It was shown by experiment that animals with the vagi cut behave in a manner similar to those which have been atropinized.

The methods employed were exactly similar to those used by Dixon and Halliburton, with the exception that we atropinized the animals instead of cutting the vagi. In addition instead of using a fresh solu-

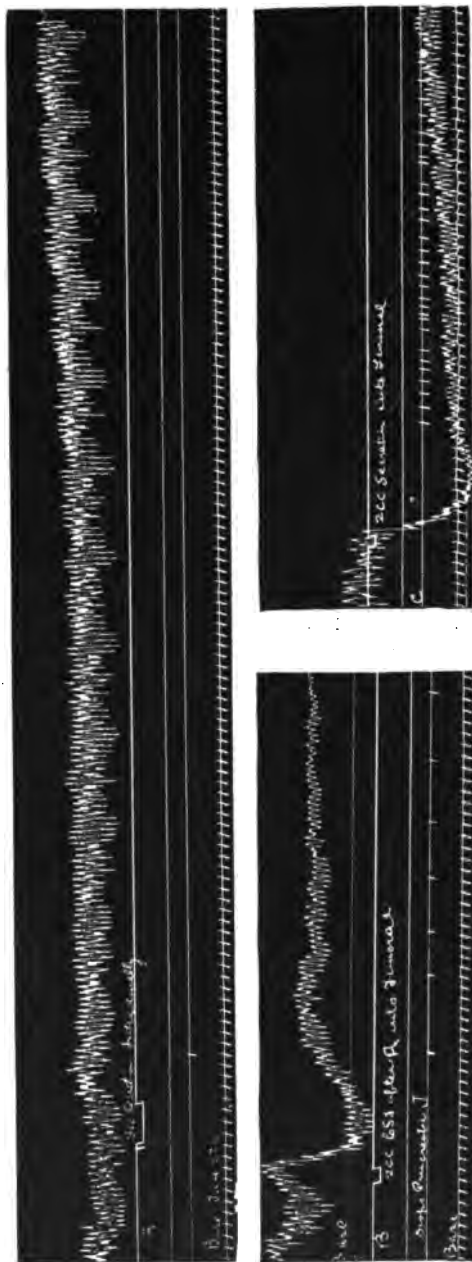


Fig. 22. This figure shows the difference in the rate of absorption of secretin intradurally and intravenously. Note that *A* shows the effect of the intradural injection of 3 cc. of neutralized secretin solution with no response from the pancreas although 12 minutes elapsed before the end of this tracing. Note in *B* the response from the intravenous injection of 2 cc. of the spinal fluid from this animal, showing clearly that the secretin was in the canal. Note in *C* the response from the intravenous injection of 2 cc. of the original secretin solution.

tion of the drug for the intravenous injection, we frequently withdrew some of the cerebrospinal fluid after the intradural injection of adrenalin and injected that intravenously, because by this method it was rendered certain that the drug was in the canal. The number of experiments performed by these men must have been small, otherwise they would have noted and corrected their error. We did not follow the long-continued action of intradural injections of adrenalin in enough animals to prove or disprove Auer and Meltzer's findings. We believe them correct. In one case we followed for  $6\frac{1}{2}$  hours the blood pressure

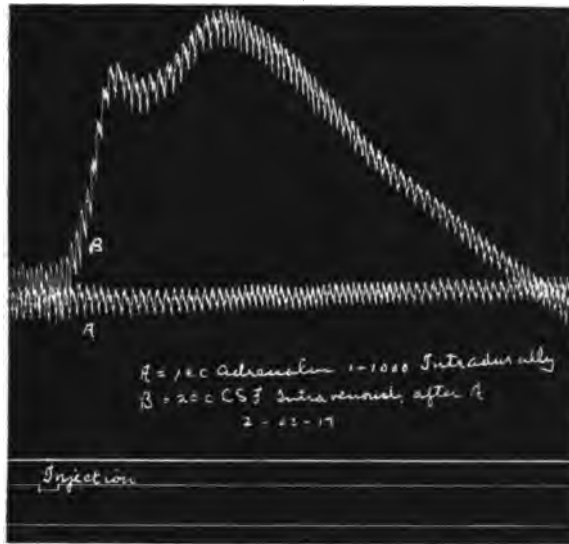


Fig. 23. This figure shows the effect of: A the injection of 1 cc. of adrenalin chloride intradurally; B the effect of the injection of 2 cc. of cerebrospinal fluid from the same animal intravenously. The fluid was withdrawn after it had been in the canal 8 minutes. B was made 10 minutes after A.

was practically constant throughout. The result of the other is shown in figure 24.

In proof of our conclusions that adrenalin and nicotone do not produce a rise in the blood pressure when injected intradurally we submit the following tables and graphs. The tables include the observations made on *all* the animals used in this particular part of the work. The graphs are the results of a few of the experiments. The effect of the intradural injection of adrenalin is shown in table 24 and figures 23 and 24.

As will be noted in the data, in only one case was the intradural injection of adrenalin followed by a marked rise in the blood pressure and in that case the latent period was longer than on intravenous injection and the fluid was bloody. A similar rise occurred in another case which was observed in some other work.

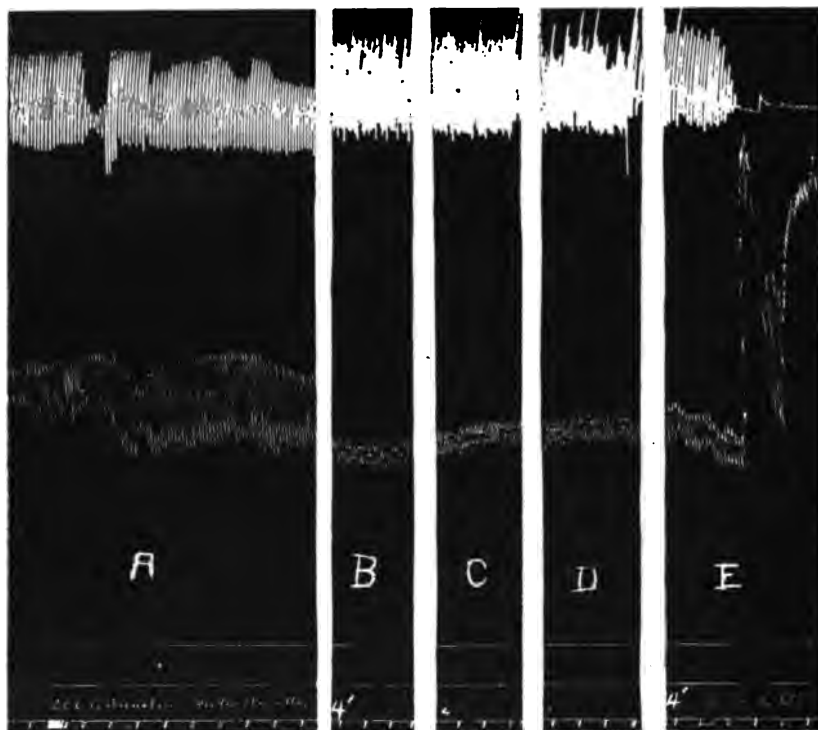


Fig. 24. This figure shows a long time (about 20 minutes) experiment after the intradural injection of 2 cc. of adrenalin chloride (1-1000). Four minutes elapsed between A and B, between B and C; and C and D. No rise in blood pressure occurred. After D was completed 8 cc. of the fluid of the animal were withdrawn at the occiput and injected intravenously, as shown in E, with a typical adrenalin effect on the blood pressure.

The effect of intradural injection of nicotine is shown in table 24 and figure 25.

In the case of the thirteen injections of nicotine in not a single case was there the characteristic rise in the blood pressure.

As will be noted in every case where the experiment is free from criticism, adrenalin and nicotine injected intradurally produced no charac-

TABLE 23

*This table shows the effect on the circulation of intradural injections of adrenalin chloride (P. D. & Co.), also the effect of intravenous injection of adrenalin chloride or of the cerebrospinal fluid of the animal after intradural injection. Tracings 1 and 2, dog #1, were from a dog without atropine with the vagi cut*

DOG	TRACING	INTRADURAL INJECTION		LATENT PERIOD OF INTRAVENOUS INJECTION WITH TYPICAL EFFECT	
		Effect	Observation period		C. S. Fluid
			minutes	seconds	
1	1	None	4	36	8.3
1	2	None	4	36	4.6
2	1	Slight rise	4	36	9.4
2	2	None	4	36	11.1
3	1	Slight fall	9	37	10.0
3	2	Slight fall	10	0	13.3
3	3	Slight rise	10	0	10.0
4	1	None	7	30	10.0
5	1	None	7	0	12.0
6	1	Slight fall	8	0	12.0
7	1	Slight fall	14	0	7.5
7	2	Slight fall	14	0	10.3
8	1	Slight rise	4	0	13.0
9	1	Slight fall	2	15	11.6
10	1	Great rise*	5	30	8.3
11	1	None	3	0	26.0
12	1	None	3	0	10.0
13	1	None	3	0	11.0
13	1	Slight rise	2	40	22.0
14	1	None	2	30	5.0
15	1	None	2	0	8.4
16	1	None	2	3	13.3
17	1	Slight fall	4	0	10.0
18	1	Slight rise	2	0	20.0
19	1	Slight fall	2	40	10.0
20	1	Slight fall	4	0	15.0
21	1	Slight rise	7	0	3.0
21	2	None	7	0	5.0

\* The fluid from this dog was bloody.

teristic rise in the blood pressure; and in every case the injection of the fresh drug or of the fluid withdrawn from the canal and injected intravenously produced characteristic results upon the blood pressure.

Therefore the absence of effect after subdural injection is due to the lack of central action of the drugs and to a lack of absorption from the space; it is not due to a failure to inject the drug into the canal, to inactivity of the drug or to a failure of response on the part of the animal. The effect of the intradural injection has already been discussed.

After proving conclusively that adrenalin intradurally had no effect upon the blood pressure comparable to the effect intravenously, the work of determining the length of the stay of the drug in the canal was

TABLE 24

*This table shows the effect on the circulation of intradural injections of 0.025 per cent nicotine, also the effect of the intravenous injection of the same drug, or the cerebrospinal fluid of the animal drawn after the drug had been permitted to act for some time*

DOG	TRACING	INTRADURAL INJECTION		LATENT PERIOD OF INTRAVENOUS INJECTION WITH TYPICAL RESULTS	
		Effect	Observation period		C. S. Fluid
			minutes	seconds	
3	3	None	10	0	13.3
4	2	None	7	30	14.4
5	2	Fall*	7	0	17.0
6	2	Slight fall	2	40	12.0
9	2	Slight fall	1	24	6.3
10	2	Slight rise	4	10	17.0
11	2	Slight fall	2	40	10.0
12	2	Slight fall	2	30	15.0
13	2	Slight fall	1	40	12.0
14	2	Slight fall	2	0	9.2
15	2	Slight fall	1	40	13.3
16	2	Slight fall	2	0	10.0
20	2	Fall*	4	0	13.3
					10.0

\* The fall in blood pressure in these cases amounted to about 30 mm. of mercury. In all the other cases the fall was insignificant and transient.

begun. The work was done in two steps. In this series four dogs were used. From each of four dogs, two under ether and two under morphine, 3 cc. of fluid were drawn, and the same amount of adrenalin chloride (P. D. & Co.) introduced. At 30 minute intervals for two hours 1 cc. of fluid was withdrawn and tested by intravenous injection into a dog. In each case the pressure of adrenalin was demonstrated by a typical rise in the blood pressure on the intravenous injection of the fluid. These experiments prove that adrenalin remains in the canal for at least two hours.

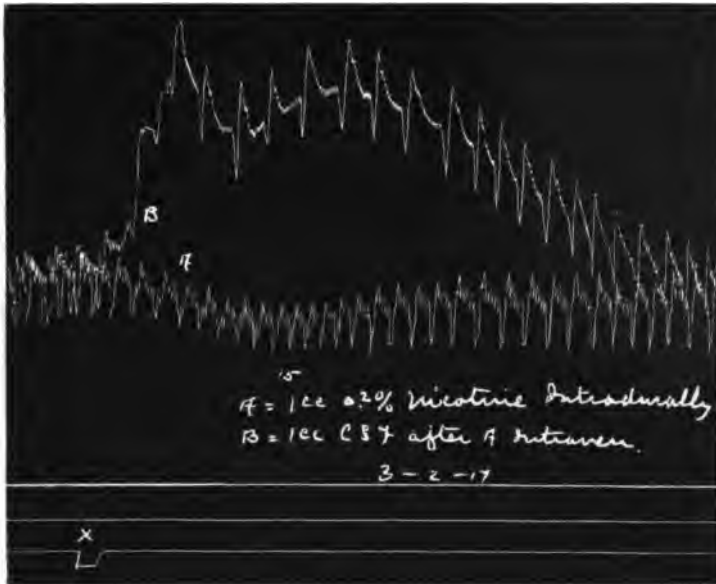


Fig. 25. This figure shows the differences in the reaction of the blood pressure to nicotine injected intradurally, and to the cerebrospinal fluid of the same animal injected after withdrawal from the canal. At A, 1 cc. of 0.2 per cent nicotine was given intradurally. At B (15 minutes later), 1 cc. of the cerebrospinal fluid from this animal was given intravenously. Injections were made at X.

In the second step of the experiment, three dogs were used. The details of one experiment were enough to show the results of the group:

*Dog A 31, weight 15 kilo*

- 8: 20 Animal anesthetized.
- 8: 24. Needle inserted into the *cisterna magna*; 5 cc. of fluid were withdrawn, and 5 cc. adrenalin chloride (P. D. & Co.) injected. No spasms. Animal quiet during recovery.
- 8: 59. Animal fully recovered. Drowsy.
- 11: 20. Animal re-anesthetized. Tracheal cannula inserted.
- 11: 24. 1 cc. of cerebrospinal fluid drawn. Fluid clear.
- 12: 24. 1 cc. of cerebrospinal fluid drawn. Fluid clear.
- 1: 24. 1 cc. of fluid drawn. Fluid slightly red in color. No blood.
- 2: 24. 1 cc. of fluid drawn. Fluid slightly red in color. No blood.
- 3: 00. Animal killed with ether.

*Dog A 32, weight 5 kilo*

2:30. Animal anesthetized.

2:45. Tracheal cannula and blood pressure cannulae inserted.

2:45 to 4:00 p.m. Fluids from dog A 31 injected one after the other.

This experiment shows conclusively that adrenalin is present in the fluid for five hours. The fluid drawn at the sixth hour gives a doubtful result, for the rise is very small, practically none. This finding has been confirmed exactly by experiment in two other animals. Therefore,

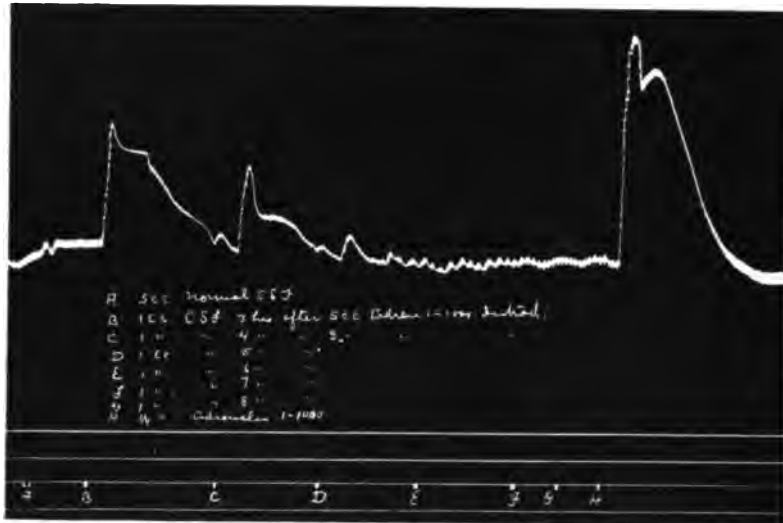


Fig. 26. This figure shows the effect of the repeated injections of 1 cc. of cerebrospinal fluid from dog A into dog B. The fluid was drawn at varying intervals 3 to 8 hours after 5 cc. of the cerebrospinal fluid of dog A had been replaced by adrenalin chloride. The graph shows a positive reaction in dog B at least 5 hours after the drug had been injected, and hence adrenalin must remain in the canal for at least that period.

adrenalin remains in the dural canal for at least five hours. The fact that adrenalin can be detected in the canal for five hours weakens the proof of Dixon and Halliburton that it passes out of the canal into the blood stream with the extreme rapidity they described in their last paper, for otherwise we might expect it all to be gone long before five hours have elapsed. (See fig. 26).

A further study was begun in order to determine the fate of adrenalin after injection of the drug into the dural canal. The possibility



that a part of the adrenalin was destroyed in the canal and thus prevented from ever reaching the blood stream was also considered. To determine whether any of the adrenalin was lost by this method mixtures of adrenalin chloride and cerebrospinal fluid were made. It was estimated that in a large dog there might well be 50 cc. of the fluid in the canal. So if 5 cc. were drawn and replaced by adrenalin chloride, the ratio of 5 in 50 or 1 to 10 of adrenalin chloride and cerebrospinal fluid would be obtained. With that conclusion in mind we made the following mixtures:

- (1) 0.1 cc. adrenalin chloride 0.1 cc. of cerebrospinal fluid 1 to 2.
- (2) 0.1 cc. adrenalin chloride 0.9 cc. of cerebrospinal fluid 1 to 10.
- (3) 0.1 cc. adrenalin chloride 1.9 cc. of cerebrospinal fluid 1 to 20.
- (4) 0.1 cc. adrenalin chloride 2.9 cc. of cerebrospinal fluid 1 to 30.

These tubes were incubated for 7 hours and 15 minutes at 37.5°C. At the end of that time the contents of the tubes were made up to 2 cc. in each case, and injected intravenously into a pithed cat, one after the other. The results are shown in figure 27.

As is seen in the figure, the mixture in tubes (1) was almost inactive, (2) showed considerable activity, (3) showed still more activity, and (4) was most active of all, because it can be seen that the curve is flatter on top. However, all were less active than 0.1 cc. of adrenalin without incubation, and less active than the same amount of the drug incubated in 1.9 cc. of a NaCl solution. Dilution of a mixture of 0.1 cc. of adrenalin and 1.9 cc. of the fluid by the addition of 1 cc. of salt solution before being incubated did not affect the destruction of adrenalin. We have here a peculiar phenomenon in which it is evident that small amounts of the fluid produce the destruction of adrenalin, while larger amounts produce less destruction of the drug. A parallel series with blood substituted for the fluid showed that small amounts of fluid produced greater destruction of adrenalin than an equal amount of blood. Here is a problem requiring more work to determine the factors involved. If the 1 to 10 ratio is correct, it is unnecessary that more than one-half of the adrenalin be absorbed, to account for the loss of adrenalin from the canal, for in that ratio at the temperature of the body, most of the adrenalin in the mixture of fluid and adrenalin is destroyed *in vitro*. This is shown by the fact that a mixture of fluid 0.9 cc. and adrenalin 0.1 cc. after incubation for 7½ hours produces only one-fourth the rise produced by such a mixture before incubation, and about one-third the rise produced by the same amount of adrenalin incubated with

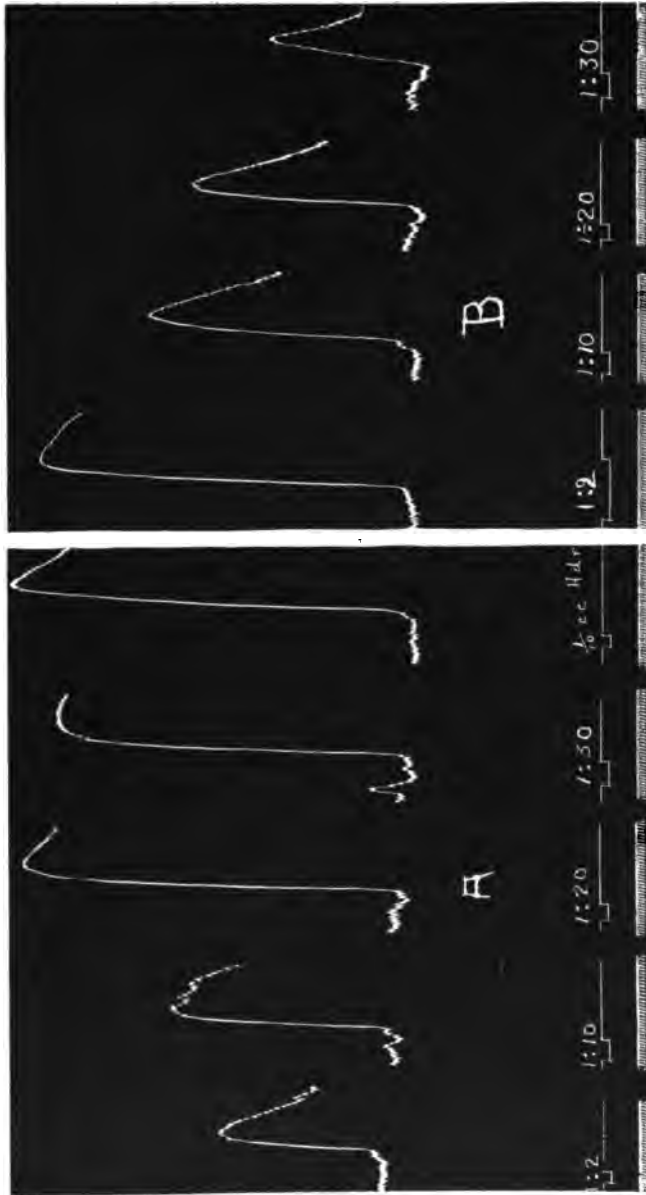


Fig. 27. This figure shows the effect on the blood pressure of a pithed cat of the injection of 10 cc. of adrenalin which has been incubated for 7 hours with varying amounts 10 cc. to 10 cc. of cerebrospinal fluid of a dog. Note in A that the greater the dilution with fluid the greater was the amount of adrenalin remaining after this period of incubation. Note in B that with salt solution, the opposite is the result, the greater the dilution with salt solution the less was the amount of adrenalin remaining after this period of incubation. The change was probably one of oxidation for the solutions were appropriately discolored.

blood. It is evident that in an experiment, uncomplicated by intravenous injection, simultaneous with the intradural injection, the absorption from the canal is slow, so not more than one-half of that injected need be absorbed to account for the loss from the canal, the rest having been destroyed by the fluid. Meltzer (34) noted the destruc-

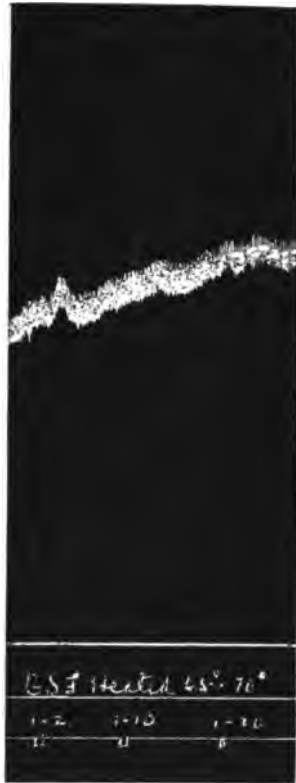


Fig. 28. This figure shows the effect of heating to 65° or 70°C. the cerebrospinal fluid, before mixing it with the adrenalin, upon the reaction shown in figure 27. The "oxidizing power" remains, but the protective power is lost so that the dilution of adrenalin with fluid in any amount results in the destruction of adrenalin in all dilutions.

tive power of fluid for adrenalin but failed to note the protective power of the larger doses.

**Conclusions:** Adrenalin and nicotine are not adsorbed from the dural canal rapidly enough to produce characteristic vascular responses. Hence the findings of Dixon and Halliburton regarding the rate of ab-

sorption are clearly in error. This error is due probably to faulty technique in the injection of the adrenalin intradurally and to the observation of too small a number of animals.

Adrenalin injected into the subdural space can be detected for at least six hours by its effect on the vascular system of the dog. It can be shown by a test experiment that relatively large amounts are destroyed by the cerebrospinal fluid at 37.5°C. in that time, hence if allowance is made for loss by destruction, relatively little of the fluid need be absorbed to account for the disappearance of the drug from the canal.

Small amounts of the cerebrospinal fluid are more active in destroying adrenalin chloride (P. D. & Co.) than large amounts.

Since substances with supposedly small molecules like adrenalin and nicotine do not pass readily from the canal, it was hardly considered necessary to study the passage of less diffusible substances like secretin and peptone to warrant us in the statement that the conclusion of Dixon and Halliburton that the transfer must be by diffusion is not warranted by the facts, for none of the substances pass out rapidly. However, a series of experiments on the intradural injection of secretin was carried out. In no case was there any secretion of pancreatic juice after intradural injection, although the preparation used was active after intravenous injection. The fluid drawn after allowing 12 minutes time for absorption was also active. (See fig. 22.)

Since the conclusions are based on faulty observations there is, from Dixon and Halliburton's work, no evidence regarding the location or mechanism of absorption.

#### V. THE EFFECT OF DRUGS ON ARTERIAL, VENOUS AND FLUID PRESSURES

##### *Drugs stimulating the secretion of glandular organs*

*a. Pilocarpine. Literature:* Of all the drugs having the power of stimulating the secretory mechanism of animals to activity, pilocarpine possesses this action to the highest degree. To a slighter degree this power is shown also by eserine, nicotine and arecolin. Thus if a secretory response on the part of the mechanism forming the fluid is to be expected in any case, it is to be expected of pilocarpine.

Cappelletti (2) studied the outflow of fluid by the fistula method and concluded that pilocarpine stimulated the outflow of fluid.

Dixon and Halliburton (11) in their first paper were very doubtful in regard to the stimulating action of pilocarpine on the mechanism forming the fluid, and listed this drug among those where the true result might be masked by respiratory or vascular changes.

These same authors (12) in their second article concluded that pilocarpine had a direct stimulating effect upon the mechanism forming the fluid, for they believe to have shown the fluid pressure to be independent of the cerebral venous pressure.

TABLE 25

*This table shows the effect of the injection of pilocarpine upon the various pressures under consideration. Note that the behavior of the pressures in the last three experiments is not materially different from the others, although the jugulars had been tied*

	ARTERIAL				VENOUS				CEREBROSPINAL FLUID			
	B	D		A	B	D		A	B	D		A
		1	2			1	2			1	2	
1	156	130	200	152	82	95	113	85	128	129	159	117
2	100	74	140	108	52	52	84	50	51	51	81	50
3	96	8	162	118	106	162	192	102	105	146	180	102
4	170	38	196	158	150	179	247	178	136	152	187	128
5	126	78	148	100	121	126	156	101	126	116	159	109
6	160	20	124	110	129	125	155	147	164	160	194	186
7	108	44	110	92	71	54	81	60	56	44	64	57
8	108	90	134	108	65	79	92	65	178	143	194	175
9	116	80	140	120	210	120	258	104	178	110	223	99
10	152	100	160	136	131	122	134	116	98	83	97	84
11	152	54	204	152	185	160	370	240	95	80	200	100
Average...	131.2	65.1	156.1	123.1	118.3	115.8	171.1	113.4	119.5	110.3	158.0	109.7
Experiments with the jugular veins ligated												
12	150	124	196	160	203	205	309	225	190	196	286	210
13	114	100	118	112	123	99	119	110	168	156	167	155
14	130	96	140	124	201	146	213	189	148	84	160	139
Average...	131.3	106.6	151.3	132.0	175.6	146.6	213.6	174.6	168.6	145.3	204.3	168.0

1 = early.

2 = late effects of the drug.

*Experiments:* The results upon the cerebrospinal fluid pressure are shown by table 25, which gives the results obtained from fourteen injections into dogs. A typical graph is shown in figure 29.

As can be seen from the table there may be in some a preliminary slight fall in venous pressure during the early stages of the action of a moderate dose of the drug, but this gives place to a marked rise in ve-

nous pressure during the depressor phase of the action of the drug. (See fig. 29.) The venous rise decreases, but persists during the arterial rise which follows the typical moderate dose, and then falls along with the arterial pressure. The fluid pressure follows the venous by the

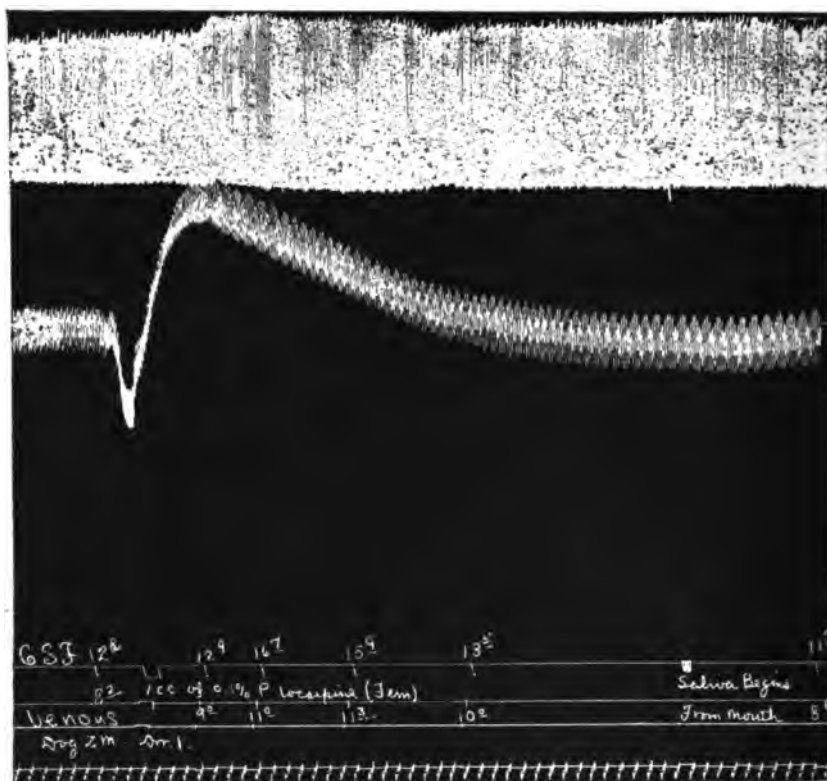


Fig. 20. This figure shows the effect on arterial, venous and fluid pressures of the injection of a small dose of pilocarpine. Note the parallel rise from the first of venous and fluid pressure. The arterial pressure fell, then rose, and then returned to normal.

manometer method. This is the usual result, and thus during the period of increased venous pressure, although arterial pressure is less than normal, an outflow fluid can be expected in the typical case. Anyone who has worked on the fluid is fully aware of the fact that the outflow does not always appear.

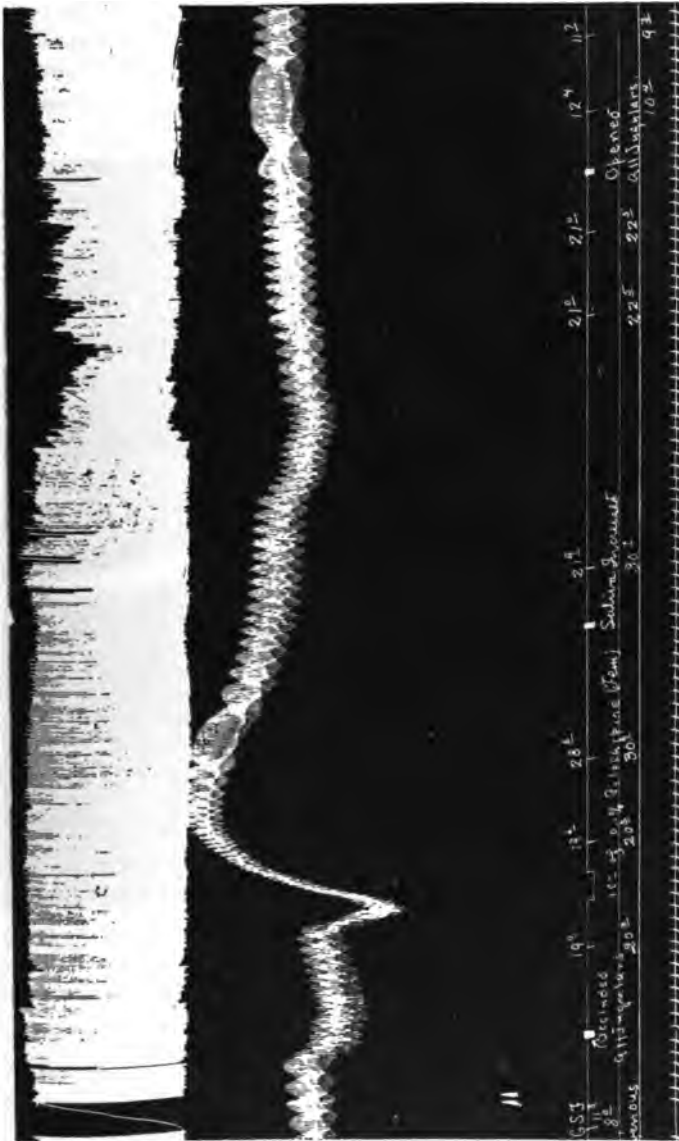


Fig. 30. This figure is from the same dog as figure 29. Conditions are exactly as in that figure except that the jugular veins were ligated. Note that the results are the same. Both cerebrospinal and venous pressures return practically to the secondary normal.

A glance at figure 31 shows what occurs in these cases. Where the dose is large, or the animal is especially susceptible to the action of the drug, a marked fall in venous pressure occurs, a fall which is paralleled by a decrease in fluid pressure. The cerebral venous and fluid pressures rise synchronously later after the cardiac depression has passed off.

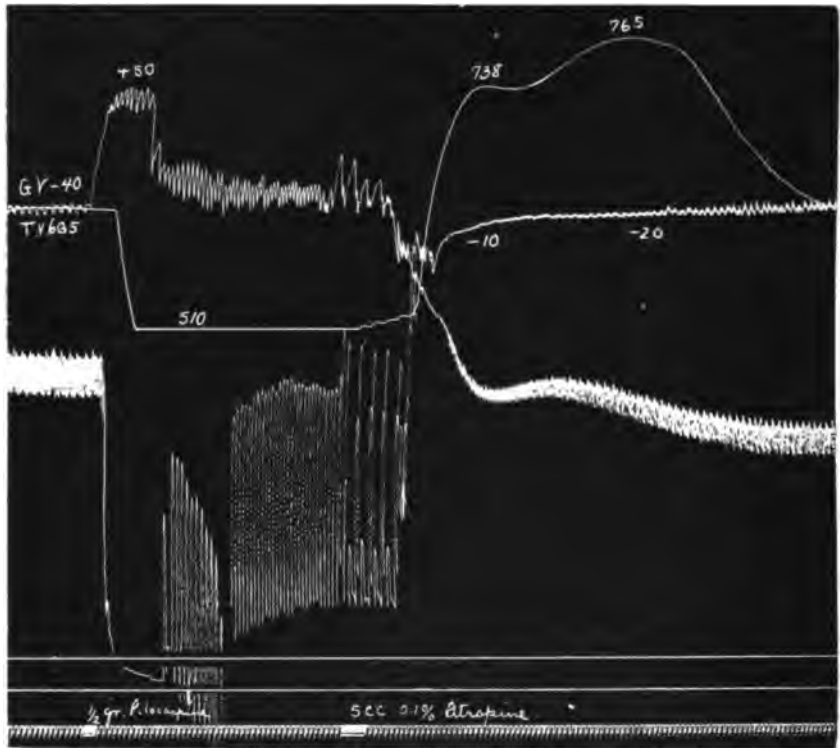


Fig. 31. This figure shows the effect of a large dose of pilocarpine, with powerful vagus inhibition. Note that torcular pressure fell while general venous pressure rose. The total fall in torcular venous pressure is not recorded owing to the rubber coming in contact with the bottom of the tambour.

Since the primary action in the case of pilocarpine is due to the vagus stimulation, any result described under the action of the peripheral vagus may be seen early in the action of pilocarpine. The later action may be markedly different, depending on the action of the drug on the vasomotor center.



Naturally since we hold that the fluid pressure is so markedly influenced by venous pressure, and since alterations in the venous pressure are sufficient to explain all the changes in the fluid pressure we are of the opinion that stimulation of the secretory mechanism need not be assumed to explain all the changes in pressures following the administration of pilocarpine.

On this point we agree with Dixon and Halliburton's first statement regarding the action of pilocarpine on fluid formation. If it be granted—and it is undoubtedly true—that arterial blood pressure does influence the fluid pressure very markedly in some animals, not at all in others, another explanation of these authors' figure 19 is that the preliminary fall in fluid pressure was due to the fall in arterial pressure in spite of the rise in venous; the rise in fluid pressure was due to the rise in arterial in spite of and not because of the fall in venous. However, we saw no changes of this type in our many experiments. In our experience fluid and venous pressures run uniformly parallel. In some cases they varied with, and in others against the arterial pressure. That torcular pressure does not always rise with marked cardiac inhibition—as they assume always to be the case—is seen clearly in figure 31.

If further evidence is needed to prove this point we offer the following evidence: In table 25 it will be noted that in experiments 12, 13 and 14 the results are in no way different from those seen in the preceding eleven experiments. The pressures fell, then rose above normal, and then returned to or below normal. The same period of time elapsed in these that elapsed in the earlier experiments. Yet these were made on animals with the jugular ligated. If new fluid were formed under the influence of pilocarpine the result logically to be expected under the conditions of the experiment would be a delay of the return of the fluid pressure to normal until after the veins were released, for all the modern work tends to show that absorption is along the vein. As can be seen from the table and by comparison of figure 29 with figure 30, both of which are from the same dog, it can be seen that the curves of pressure were exactly the same whether the jugulars were or were not ligated before the experiment. As can be seen from figure 30, the ligation of the jugulars produced an initial rise in both pressures, the pilocarpine curve was then superimposed upon this rise, the pressures returned to the new normal in the normal time and then returned to near the original normal when the veins were released. It is thus perfectly obvious that all the changes to be observed in the behavior of the cerebrospinal fluid find an explanation in the mechanical

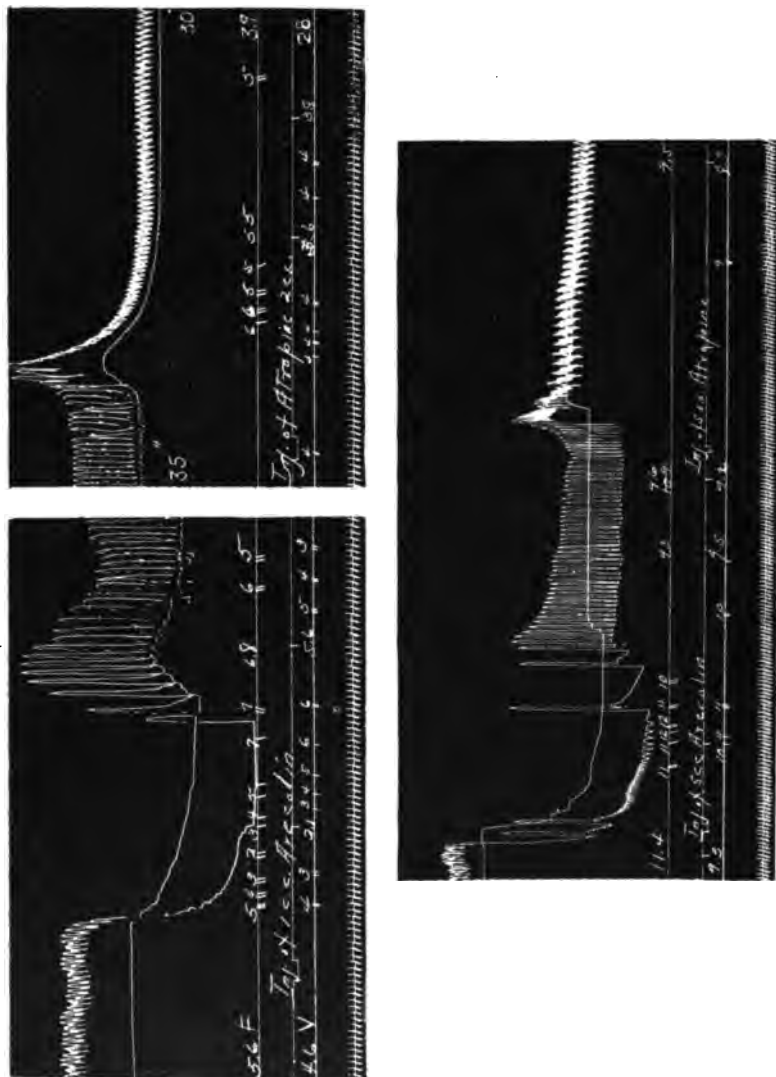


Fig. 32. This figure shows the effect of arecoline on all the pressures.

changes due to changes in arterial and venous pressure in the skull. Not only is there no need of ascribing the changes to secretion but there is positive evidence *against* the secretion of cerebrospinal fluid under the influence of pilocarpine. We believe that changes in the venous and arterial pressure are sufficient to explain the changes seen in fluid pressure and in fluid outflow when pilocarpine was injected.

*b. Arecoline.* Arecoline produces the changes to be expected if these changes are due to purely mechanical causes (see fig. 32).

*c. Nicotine.* Nicotine also produces changes which can be explained on a purely mechanical basis. (See fig. 33).

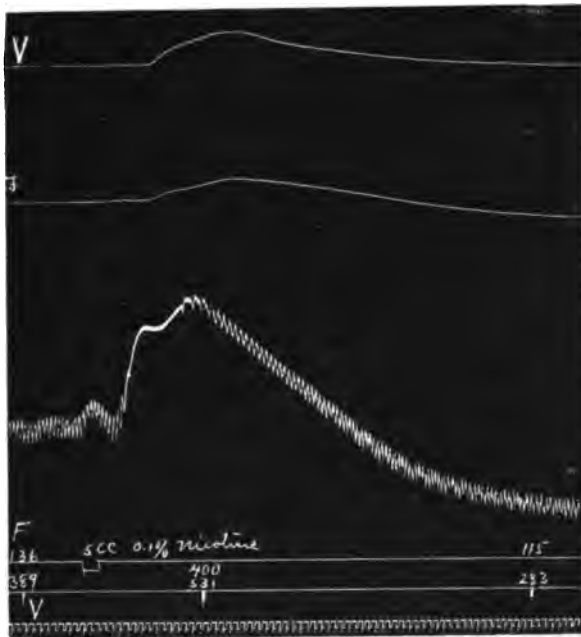


Fig. 33. This figure shows the effect of nicotine on all the pressures.

#### *Drugs inhibiting the secretion of glandular organs*

*a. Atropine.* Yoshimura (35) found microscopic changes in the choroid which he interpreted as the inhibition of fluid formation. Dixon and Halliburton (11) found with atropine an increased outflow especially if the drug was given soon after tapping the ventricle. This drug they classed among those yielding a slight increase in fluid formation.

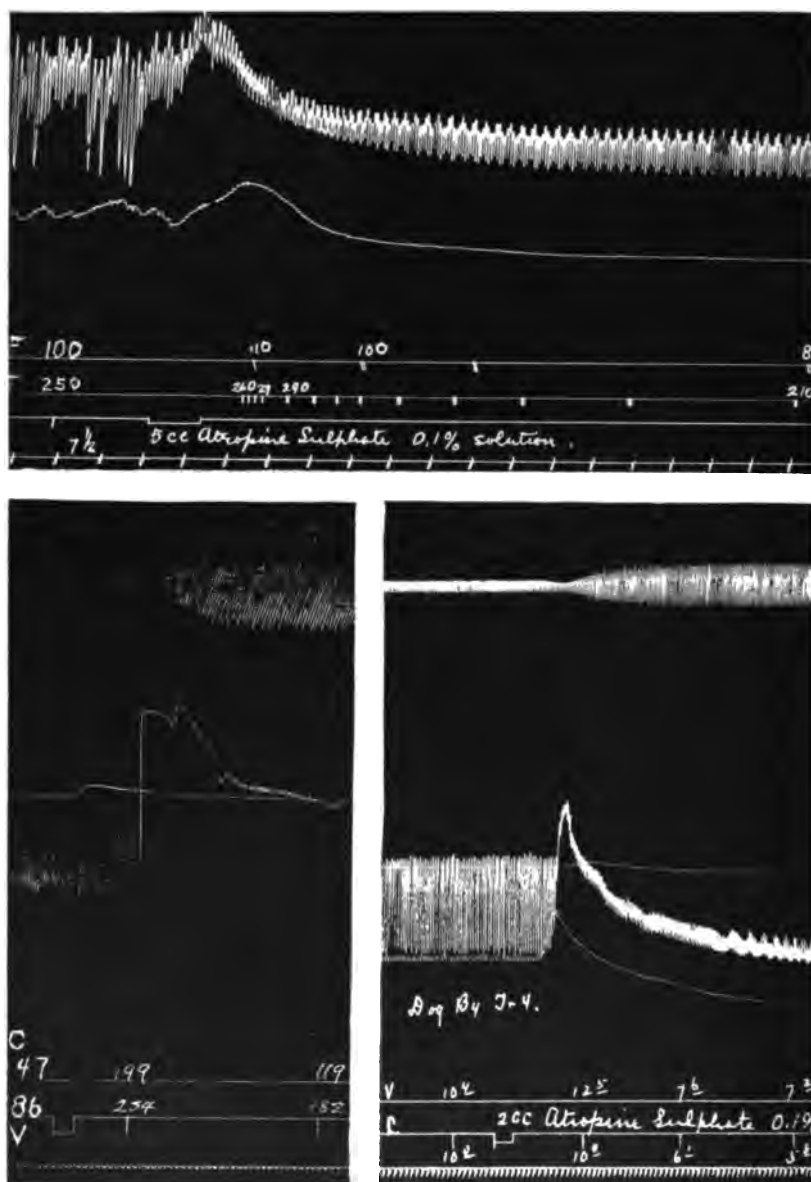


Fig. 34. This figure shows the effect of atropine on the various pressures. In each case a dose of pilocarpine had been administered previously.

Our experience with atropine is that the result is exactly what is to be expected from the alteration in the venous and arterial pressures. (See fig. 33.)

TABLE 26

*This table shows the effect of the injection of amyl nitrite upon the arterial, venous and fluid pressures. It shows also the effect of the drug upon the outflow of fluid, and the arterial and venous pressures*

	ARTERIAL			VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A
1	130	96	132	78	{ 73 89*	69	109	{ 102 108*	76
2	170	136	168	140	{ 125 155*	146	200	{ 175 214*	208
3	168	104	156	136	{ 110 140*	131	200	{ 155 204*	195
4	144	100	154	141	{ 135 150*	152	195	{ 174 241*	247
5	144	198	150	560	365	520	385	245	387
6	120	70	114	76	54	69	211	120	226
7	96	56	88	59	49	53	66	52	56
8	164	86	122	87	80	72	216	164	205
9	144	120	144	96	85	98	124	109	120
10	124	58	80	148	84	127	145	105	138
Average.....	140.4	102.4	130.8	152.1	116.0	143.7	185.1	140.1	185.8

*Outflow method*

							Drops per minute		
1	96	44	108	86	80	85	4.5	0	0
2	108	54	110	88	82	85	2	1	2
3	126	92	120	205	186	200	5	0	1
4	164	130	170	153	129	154	2	1	2
5	158	114	166	120	{ 100 128*	120	3	{ 0 6*	2—
6	116	80	40	566	500	579	1—	19	1—
7	180	140	180	136	120	136	5	5	3
8	120	60	110	220	192	208	0	20	0
9	110	56	100	208	192	200	0	21	0
10	140	98	130	421	397	412	0	0	0
11	118	96	110	468	440	462	42	6	30
Average.....	130.5	87.6	122.2	242.8	219.8	240.1	5.8	6.6	3.7

\* In these cases a secondary pressor effect followed the primary depressor effect, and showed itself clearly in both the venous and fluid apparatus.

*Drugs having no effect on the secretion of glandular organs*

a. *Amyl Nitrite*. As is well known amyl nitrite is an active vasodilator, and as such might be expected to produce rather marked effects upon the fluid pressures.

Dixon and Halliburton (11) in their first paper ascribed to amyl nitrite a slight stimulating action upon the mechanism generating the fluid. They believed that this effect was produced largely by asphyxia. In their second paper these same authors state (p. 151): "The rise in C-S pressure has certainly all the characteristics of an independent

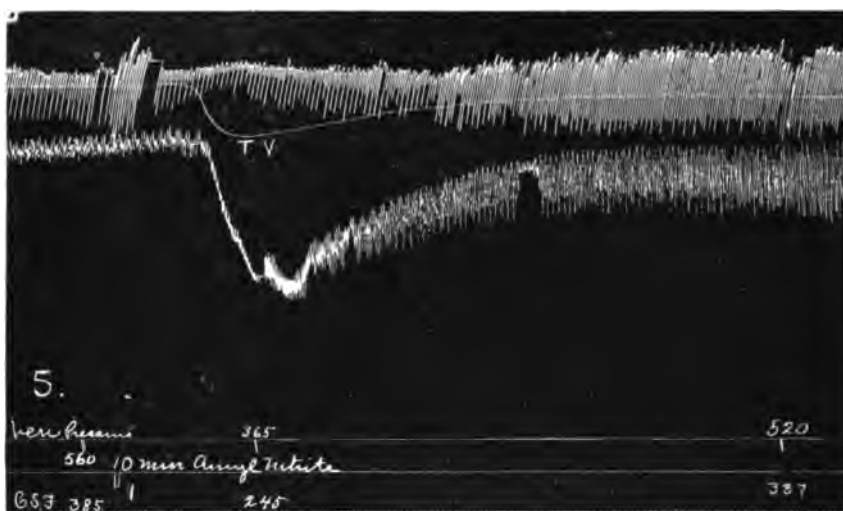


Fig. 35. This figure shows the effect on all the pressures of the injection of amyl nitrite. Note that all pressures fell.

factor," which we interpret to mean that they believe fluid formation to be stimulated by the drug. They show a tracing in which the rise in fluid pressure was large out of all proportion to the rise in venous pressure. At the same time the arterial pressure fell.

In table 26 we show our results in ten experiments by the manometer method, and in eleven experiments by the outflow method. Graphs of typical experiments are shown in figures 35 and 36.

As can be seen from our tables and figures, the effects of amyl nitrite on venous pressure are, in our experience, by no means the effects shown by Dixon and Halliburton. They state: "This effect (increased fluid

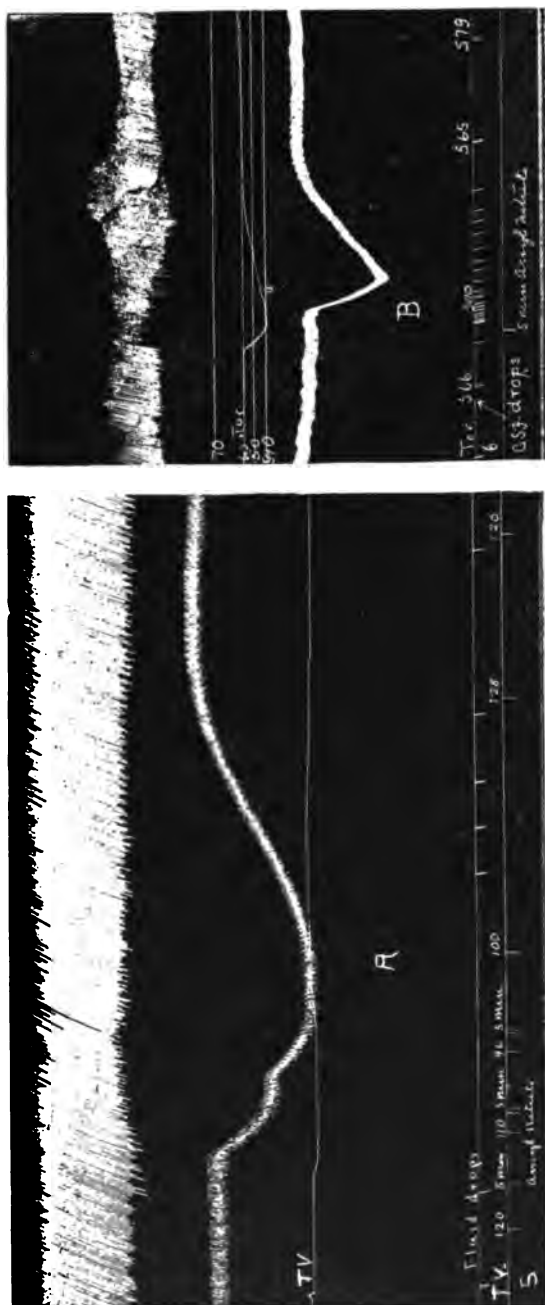


Fig. 38. This shows the differences in the reaction of different animals to the same drug, amyl nitrite. In both cases there was a fall in both arterial and venous pressures; while in A there was an almost complete cessation of the drops of fluid, in B there was a marked increase in the rate of flow.

outflow) is often associated with a general rise in venous pressure including torcular pressure." In *every one* of the twenty-one cases which we show in our table there was a fall in torcular pressure. In five of the twenty-one there was a secondary rise above the normal. It is to be noted also that in our table the fluid pressure ran parallel with the venous pressure. Sometimes it was higher, sometimes lower, but always it ran a parallel course. In those four cases where the venous pressure showed a secondary maximum after the fall the fluid showed a similar maximum above the normal. So much for the pressure method.

If we turn now to the outflow method it will be noted that in only four cases of eleven was there an increase in the outflow of fluid on the injection of amyl nitrite. In one of these (5) the increase was preceded by temporary decrease. The decreased outflow occurred during decreased venous pressure. It must be pointed out that an increase comes frequently by this method with both venous and arterial pressures below normal. (See expts. 6, 8 and 9, table 26, and fig. 36.) How there can be an increased outflow with falling arterial and venous pressure has already been explained. The increased outflow is due to increased leakage of fluid from the cerebral cavity into the medullary region.

Our conclusion regarding the action of amyl nitrite is that all the changes observed in the behavior of the fluid can be explained logically by changes in arterial and venous pressure. Secretion need not be assumed to explain any of the facts.

#### CONCLUSIONS

1. The manometer method is superior to the outflow method as a means for studying the cerebrospinal fluid.
2. It is essential that arterial, venous and fluid pressures be measured simultaneously and continuously, if trustworthy evidence regarding the mechanism of fluid formation is to be obtained.
3. Venous and fluid pressures, while positive in normal animals, are always less than arterial pressure. Venous and fluid pressures are almost, but not exactly equal; no law can be given in regard to which is under the greater pressure.
4. Venous and fluid pressure vary in the same direction, and to some degree proportionally in nearly every case; these pressures may or may not vary in the same direction as the arterial pressure.



5. Raising the venous pressure raises the fluid pressure; lowering the venous pressure lowers the fluid pressure. Therefore the measurement of the venous pressure is absolutely necessary to rule out venous pressure changes, where the study of the fluid is under consideration.

6. Increasing or decreasing the fluid pressure moderately does not alter the venous pressure unless the arterial pressure is affected.

7. Ligation of the arteries entering the circle of Willis produces only passive changes in the skull characterized by a fall in the arterial, venous and fluid pressures, with almost perfect readjustment when the vessels are released.

8. Ligation of arteries in other parts of the body produces only mechanical changes.

9. The administration of adrenalin produces purely passive changes in the fluid pressure, although rapid outflow follows such an injection if the outflow method of study is employed. The fact that ligation of the jugulars does not modify the results either as regards the form or the duration of the curves of pressure change, is additional evidence that there is no new formation.

10. Arterial pressure can modify the fluid pressure independent of the venous pressure.

11. The fluid pressure is the result of at least two factors: The influence of the venous pressure, plus the influence of the arterial pressure; thus the fluid pressure in some animals will be higher than the venous pressure.

12. The effect of the peripheral vagus upon the fluid pressure is exactly what is to be expected, if the changes are due to mechanical causes. Increased secretion due to asphyxia need not be assumed to explain the results.

13. The greater the amount of fluid there is in the canal the more nearly does the fluid rise and fall equal the venous rise and fall following adrenalin; hence the animal with a large amount of fluid in the canal will show greater alterations in the fluid pressure during an experiment.

14. Increasing the active respiratory movements by stimulation of a sensory nerve like the sciatic, produces the changes in the fluid pressure to be expected from the changes in arterial and venous pressure. The same stimulation may produce either increased or decreased outflow of fluid, with the former the more common.

15. Inhibiting the respiratory movements by stimulation of the superior laryngeal or the central vagus raises the fluid pressure; but this

rise is adequately explained by the changes in the arterial and venous pressures.

16. The increased outflow of fluid observed during asphyxia is adequately explained by the rise in the arterial and venous pressures.

17. We were unable to reach any conclusions regarding the normal rate of the formation of the cerebrospinal fluid.

18. Adrenalin and nicotine are not absorbed from the canal rapidly enough to produce the characteristic effect upon the blood pressure.

19. Adrenalin can be detected in the canal for at least five hours after its injection.

20. Adrenalin is more rapidly destroyed *in vitro* by cerebrospinal fluid than by blood. A peculiarity in the reaction is seen in the fact that small quantities of the fluid, whether diluted or not, destroy adrenalin in solution more rapidly than do larger quantities of the fluid. This "protective action" of the larger quantity but not the destructive principle is destroyed by heating the fluid to 65°-75° for 30 minutes.

21. Secretin has no effect upon the pancreas when injected intradurally.

22. The effect of pilocarpine, when injected intravenously, upon the fluid can be explained logically by the effect of the drug upon the arterial and venous pressure in the skull. The same holds true for nicotine and arecoline.

23. The effect of atropine upon the fluid pressure can be explained logically by the effect of the drug upon the arterial and venous pressure in the skull.

24. Amyl nitrite always (twenty-one consecutive trials) produces a fall of the fluid venous and arterial pressures. The increased outflow of fluid, occasionally but by no means always observed, is due either to the accumulation at the expense of the fluid of venous blood in the sinuses with decreased venous pressure, or is due to the increased facility with which fluid passes into the region of the fourth ventricle from distant parts of the nervous system.

25. All the changes in the fluid pressure and fluid outflow which have been offered as proof of the secretory mechanism of formation of the cerebrospinal fluids can be traced to alterations in venous and arterial pressures in the skull. There is no proof that the fluid is formed by secretion.

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## STUDIES ON THE CEREBROSPINAL FLUID

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### VI. A STUDY OF THE EFFECTS OF TISSUE EXTRACTS

This series of experiments was undertaken with the view of determining what effect the injection of tissue extracts has on the cerebrospinal fluid and, particularly, to ascertain whether or not they possess a specific stimulating action, producing an actual increase in the amount of fluid produced.

It is an established fact that all tissue extracts—excepting adrenal and posterior lobe of the hypophysis—have a depressor effect on the vascular system. The amount of the depressor effect varies with the extract used—in general the extract of those organs having the largest external secretion produce the most marked effect (1). There is one exception—the extracts of nervous tissue give as decided an effect as any. In addition to the vascular effect, a number of recent workers claim a specific and direct action for certain of the extracts in causing an increase in the amount of cerebrospinal fluid, presumably by stimulation of the choroid plexus. Frazier and Peet (2) state that brain extract increases the secretion and that thyroid extract decreases the secretion from the choroid plexus, quite independent of blood pressure changes. Weed and Cushing (3) conclude that the extract from the posterior lobe of the hypophysis stimulates the choroid plexus, producing what they term a “choroidorrhea.” Dixon and Halliburton (4), (5) conclude from their work that extracts from brain, and particularly from choroid plexus, cause a decided increase in the secretion of the fluid.

### METHODS

In a previous paper (6) it has been pointed out that the conclusions set forth by these writers are open to criticism because with the methods employed, their observations were incomplete and thus subject to gross

error. In this series of experiments the manometer method, previously described, was employed throughout, the outflow method was employed only a few times to correlate our results with those of other writers. Particular care was observed in establishing venous and fluid pressures and if either of these was unsatisfactory the experiment was discarded. The circle of Willis pressure was taken in addition to the general arterial pressure but this worked well only in the larger dogs. All injections were made into the femoral vein.

*Preparation of organ extracts.* The organs and tissues used in these experiments were taken principally from dogs. The fresh tissues were removed promptly from the animal and if not used immediately were kept in the ice box. They were cut into small pieces and then thoroughly ground in a mortar with fine sand, and after being allowed to extract for about one-half hour, with either Ringer's or isotonic salt solution, they were centrifugalized. They were warmed before injection. Most of the extracts were made up to 50 per cent strength. The amount of tissue in the choroid plexus and in the hypophysis was so small that these extracts were made up so one cubic centimeter represented each choroid and each hypophysis. Aqueous humor and cerebrospinal fluid were taken at the same time that the choroid plexus and hypophysis were removed from the dogs—these fluids were used without concentration. Extracts were made of the following tissues: lymph gland, ovary, testicle, spleen, pancreas, submaxillary and parotid glands, skeletal muscle, heart muscle, kidney, liver, nervous tissue—cerebrum, cerebellum, medulla, cord and pineal body—thyroid gland, adrenal, hypophysis and choroid plexus. In addition a number of lymphogogues—urea, peptone, cane sugar and strawberry extract—were used.

For the sake of convenience in discussion and for the sake of brevity the extracts will be considered in three groups: *a*, those having a depressor effect on the vascular system—including those in which the effect was practically negligible; *b*, those having a pressor effect; and *c*, those for which a specific action is claimed, so modifying the activity of the choroid plexus that either an increased or decreased formation of the fluid results.

A careful tabulation was made of the principal changes in all the pressures caused by each of the extracts used, and thus the action of any one of them can be seen by reference to the proper table. Only three readings were recorded in the tables, though many more were taken. The first reading represents the normal pressure before injection.

tion, the second the maximum change after the injection, and the third represents the return to equilibrium. Where the changes in the arterial pressure were marked, the changes in the venous and the fluid pressures were practically always in the same direction though not exactly synchronous as to point of time—the maximum change in the latter being seen only thirty seconds to forty seconds later than the maximum change in the arterial pressure. The reason for this delay has already been discussed adequately (6). In a considerable number of cases there was an immediate and temporary rise in the arterial pressure following the injection, amounting usually to  $2\frac{1}{2}$  mm.—this was usually accompanied by a corresponding rise in venous and fluid pressures. Since these vascular changes were not constant, it was thought best to omit them from the tables—reference will however be made to them later.

#### EXTRACTS HAVING A DEPRESSOR EFFECT

The list of extracts having a depressor or a negligible effect includes those made from spleen, pancreas, lymph-node, ovary, testicle, sub-maxillary, parotid, skeletal muscle, heart muscle, kidney, liver, also fluid and aqueous humor. Thyroid, choroid plexus and brain extract likewise have a depressor action, but for reasons already stated, viz., because a specific action has been ascribed to them by some recent writers, they will be discussed separately. A study of the tables, table 1 to table 3 inclusive, shows that all of these extracts produce very similar changes—the difference being a quantitative one only. Hence the effects produced by only one of these—kidney—will be taken as representative of the entire group, and it only will be discussed in detail. The results from other extracts are shown in the tables.

*Effect of kidney extract on arterial, venous and fluid pressures.* Five injections of 5 cc. each were made of kidney extract into four different dogs. Two injections were made in one experiment and the changes produced ran practically parallel in all the pressure tubes. The first injection caused a fall of 34 mm. of mercury and of 30 mm. in circle of Willis pressure (see exper. 3). In venous pressure there was a fall of 6 mm. ( $\text{Na}_2\text{CO}_3$ —1.088 sp. g.) and in fluid pressure a fall of 6 mm. ( $\text{NaCl}$ —sp. g. 1.088). All the pressures then rose and after they were again stationary the arterial pressures stood respectively 6 mm. and 4 mm. higher, and the cerebrospinal fluid and venous pressure stood respectively 7 mm. and 23 mm. higher than the normal before the

injection. The depressor effect of the second injection was slightly more than for the first—the fall in arterial pressures being respectively 44 and 36 mm. while the fall in venous and cerebrospinal fluid pressures were respectively 25 and 11 mm. (see exper. 2 and fig. 1). Here the recoveries were, with the exception of circle of Willis pressure, to

TABLE 1  
*Extracts of kidney, ovary, testicle*

EXPERIMENT	Arterial			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
1. Kidney extract												
1	160	102	168				94	76	112	122	112	142
2	120	76	120	82	46	78	127	102	127	79	68	79
3	116	82	122	78	48	82	82	76	105	68	62	75
4	136	118	150	130	116	138	67	65	61	48	47	46
5	122	88	130	107	80	115	109	79	118	132	109	144
Average	130.8	93.2	138	99.2	72.5	103.2	95.8	79.6	104.6	89.8	79.6	97.2
2. Ovary extract												
1	122	90	132	96	66	104	50	50	54	47	49	47
2	130	96	132	104	70	104	54	60	57	48	49	49
3	156	158	160	126	127	128	111	118	124	115	120	129
4	160	158	160				124	127	122	129	131	122
5	158	143	160	142	126	140	68	69	64	48	55	49
6	126	120	118	76	73	70	251	243	241	245	236	235
7	118	112	114	70	64	66	241	240	234	235	230	228
Average	138.5	125.3	139.4	102.3	87.6	102.0	128.4	129.5	128	123.8	124.2	122.7
3. Testicle extract												
1	154	114	146				87	65	71	116	102	105
2	112	104	108				88	87	87	94	92	91
3	104	94	104	80	80	80	77	75	78	288	278	288
4	104	96	104	80	70	81	78	66	65	288	252	255
Average	118.5	102	115.5	80	75	81.5	82.5	73.2	75.2	196.5	181	184.7

exactly the same levels as those preceding the injection. In experiment 1 the falls in pressures were again practically parallel—the fall in arterial pressure was comparatively larger than in experiment 2, and the changes in venous and cerebrospinal fluid comparatively less. These latter pressures stood higher after their return to normal than

they were before the injection but the final arterial pressure also stood higher than the initial one. The changes in experiment 5 were almost an exact repetition of those in experiment 1—there was a corresponding

TABLE 2  
*Extracts of liver, spleen, pancreas, submaxillary*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
4. Liver extract												
1	100	84	100	90	78	90	109	102	99	132	128	130
2	106	64	94	72	40	64	117	84	89	65	49	58
3	156	116	160				74	68	80	106	103	107
4	114	78	108	76	48	72	123	100	117	72	58	65
Average	119	85.5	115.5	79.3	55.3	75.3	105.7	88.5	96.2	93.7	84.5	90.0
5. Spleen extract												
1	148	146	158	142	140	142	66	69	69	45	51	49
2	130	112	122	115	102	110	118	114	88	144	122	97
3	166	100	148				110	73	88	140	108	123
Average	148	119.3	142.6	128.5	121	126	98.0	85.3	81.6	109.6	93.6	89.6
6. Pancreas extract												
1	130	86	128	89		84	96	80	90	61	46	60
2	106	76	110	84	48	74	119	70	71	79	38	41
3	100	54	74	90	48	68	99	52	78	130	105	111
4	160	70	130	128	76	120	130	70	91	115	80	65
5	144	76	78				89	47	45	126	88	87
Average	128	72.4	104	97.7	57.3	86.5	106.6	63.8	75	102.2	71.4	72.8
7. Submaxillary extract												
1	118	92	120	105	90	114	97	100	104	131	130	142
2	154	96	136				118	92	106	117	100	108
3	124	90	126	100	80	96	55	63	67	52	54	62
4	150	104	154	138	104	140	61	62	63	46	52	45
Average	136.5	95.5	134	114.3	91.3	117.0	82.7	79.2	85	86.7	84	90.2

fall in each of the pressures with a recovery in each case to pressures higher than the ones preceding the injection. In experiment 4 there was a slight fall in arterial pressure with practically no change in venous



TABLE 3

*Extracts of lymph gland, parotid, skeletal muscle, heart muscle, peptone, urea and sugar solutions and strawberry*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
8. Lymph gland extract												
1	120	120	112				104	97	88	102	96	94
2	162	120	156				81	63	87	108	99	114
3	90	59	80	62	23	54	87	62	86	65	47	56
4	80	54	76	54	48	52	86	67	86	56	45	55
5	120	108	114	114	96	102	104	96	102	142	132	138
6	122	126	120	92	92	88	71	74	71	65	68	65
Average	115.6	97.8	109.6	80.5	64.7	74	88.8	76.5	86.6	89.6	80.8	87
9. Parotid extract												
1	137	80	124				105	83	107	106	82	104
2	90	58	110	52	56	86	67	60	90	189	129	245
Average	113.5	69.0	117				86	71.5	98.5	147.5	105.5	174.5
10. Skeletal muscle extract												
1	122	88	124	104	80	108	111	81	109	138	108	127
2	147	130	144				86	82	89	124	124	126
3	88	64	78	54	45	54	84	74	79	55	50	53
4	78	62	78	54	42	53	79	69	75	53	49	51
5	156	150	156	142	138	144	65	68	66	42	47	45
Average	118.2	98.8	116.0	88.5	76.2	89.7	85.0	74.8	83.6	82.4	75.6	80.4
11. Heart muscle extract												
1	112	130	120	92	102	98	110	132	119	122	142	141
2	120	114	122	99	94	104	113	102	111	141	129	138
3	120	72	122	94	58	96	98	83	105	243	170	268
Average	117.3	105.3	121.3	95.0	84.6	99.3	107.0	105.6	101.6	168.6	147.0	182.3
12. Peptone solution												
1	90	54	66	78	44	50	67	38	45	105	69	87
2	88	56	84				95	77	97	96	58	62
3	110	90	70	92	80	60	72	60	58	43	36	30
4	150	154	152	122	126	126	54	54	55	56	58	55
5	152	120	94	126	100	80	55	55	62	55	100	75
6	142	18	66	132	72	80	59	-10	36	64	-10	40
7	82	68	86	82	78	82	44	40	46	49	46	56
Average	116.2	80.0	88.2	105.3	83.3	79.6	63.7	44.8	57	66.8	51	57.8

TABLE 3—*Concluded*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
13. Urea solution												
1	110	102	106	84	78	78	82	86	88	168	173	190
14. Sugar solution												
1	118	112	120	88	84	96	112	112	112	305	295	265
15. Strawberry extract												
1	120	90	112	94	70	88	78	65	77	139	127	146

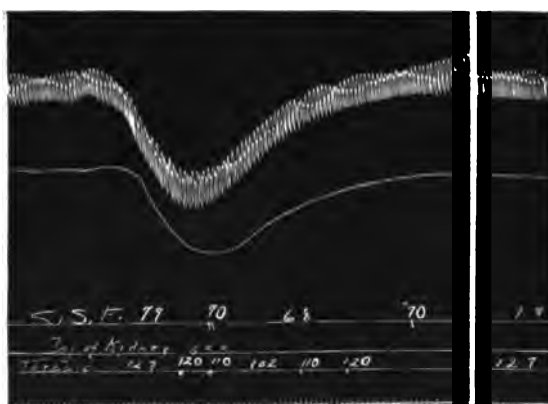


Fig. 1. This figure shows the effect of the injection of 5 cc. of kidney extract. Note the perfect return to normal of all the pressures.

and cerebrospinal fluid pressure; the final readings showed the arterial pressure to be higher and the venous and cerebrospinal fluid pressures lower than the normal pressures before the injection.

As has been stated, the changes produced by the injection of kidney extract on the fluid pressures are quite representative for all the depressor extracts. Throughout all the experiments, the correspondence between venous and cerebrospinal fluid pressure is especially to be noted. The changes are in the same direction and usually comparable in amount though never or rarely exactly equal. Where there is but little change in arterial pressure, as when aqueous humor is injected, there is little if any change in venous and fluid pressures.

However, in the vast majority of cases, where there is a marked fall in arterial pressure, there is a corresponding fall (see tables) in venous and fluid pressures. It has already been pointed out that the low points in the venous and fluid pressures are not reached synchronously with that in the arterial but follow the arterial fall by from thirty to forty seconds. The time at which the low points in venous and fluid pressure come depends largely on the change in the arterial pressure—if the arterial pressure rapidly returns to normal there is but a slight fall in

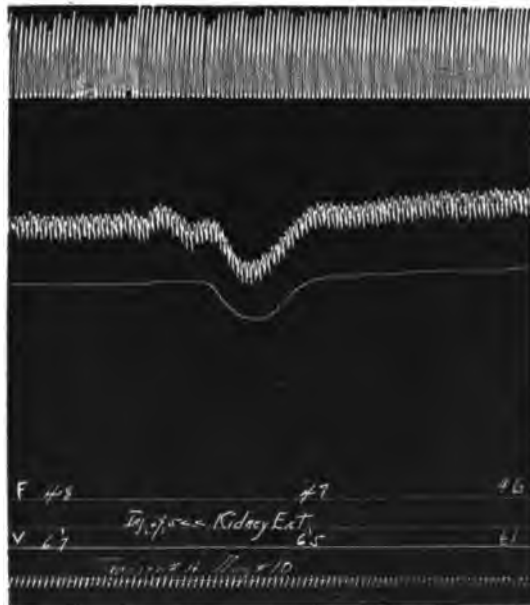


Fig. 2. This figure shows that when arterial drop and recovery occurs rapidly, the fluid and venous pressures may change together but do not follow the arterial change.

the venous and fluid pressures, following closely the change in arterial pressure; on the other hand, if the arterial pressure returns to normal slowly, then the low points in venous and fluid pressures are more marked and in point of time considerably later. In experiment 3, following the injection of 5 cc. submaxillary extract, there is a marked fall (see table 2) in arterial pressure with a return to normal in about thirty seconds. Here there is no fall in venous and fluid pressures—in fact these pressures show a very slight rise about thirty-five seconds

after the injection (fig. 3). In experiment 4, the injection of 5 cc. pancreas extract gave a decided fall in arterial pressure (see table 2) in which the return toward normal was very slow—here the lowest point in venous and fluid pressure was not reached for over two minutes. (See fig. 4). The experiments just cited are the exception rather than the rule—but they show clearly a fact which is not so evident in the average changes. The adjustment in the venous sinuses and fluid

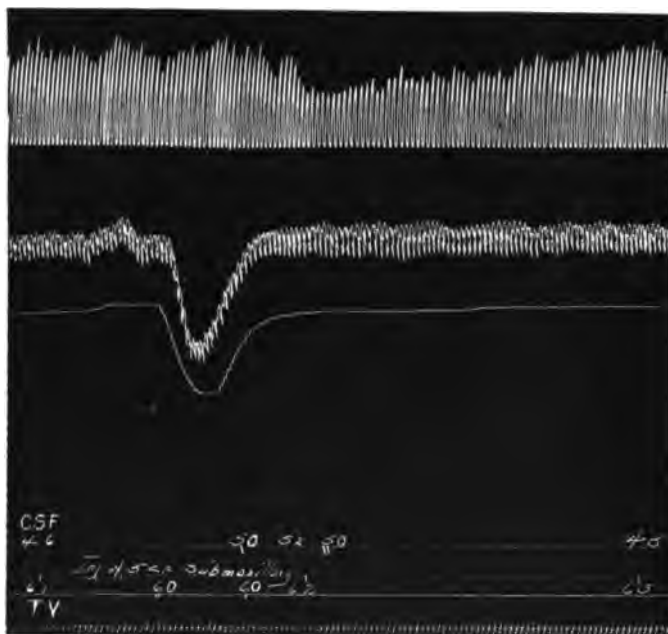


Fig. 3. This figure shows the effect of the injection of 5 cc. of submaxillary extract. Note that in this case the venous and fluid pressures rose with the fall in arterial pressure. This is different from the usual result.

spaces to changed arterial pressure is not immediate, probably because with each respiration there is a partial blocking of the various pathways of communication; hence when there is a fall in arterial pressure, some little time is required for the readjustment of the venous and fluid pressures. Thus if the return to normal is rapid as in experiment 4 (kidney, fig. 2) there is not sufficient time for readjustment in the venous and fluid pressures as there is in experiment 4 (pancreas, fig. 3) where the return to normal in arterial pressure is slow. Therefore it

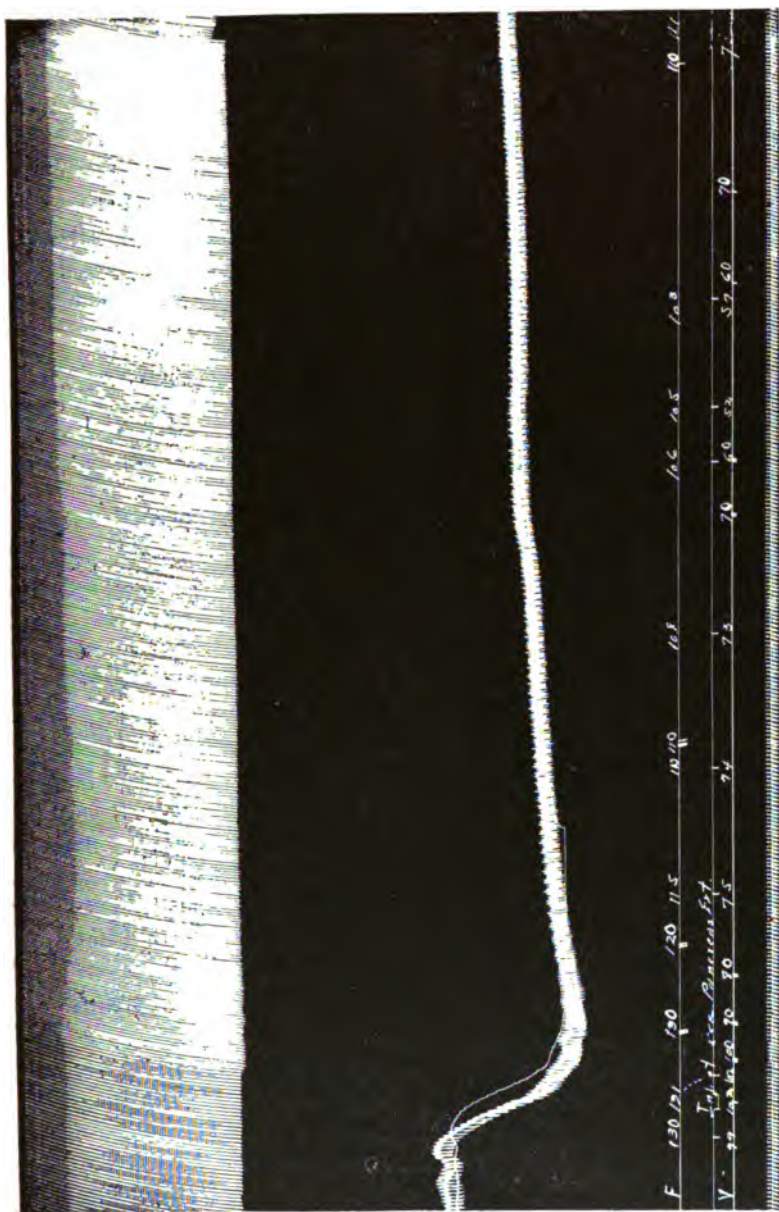


Fig. 4. This figure shows the effect of pancreas extract upon the various pressures. Note that venous pressure fell but made a fair recovery later.

is evident that the low point for venous and fluid pressures recorded in most of our experiments is not the low point which is possible with such a fall in arterial pressure since the arterial pressure commences to rise before sufficient time has elapsed to allow the venous and fluid pressures to become completely readjusted. These readjustments, which come slowly with a decrease in pressure, may show little if any delay with a rise in pressure, because the resistance offered to the redistribution of the fluid is readily overcome by increased pressure.

Our observations of the vascular and fluid changes following the injection of depressor extracts are directly opposite to those made by Frazier and Peet (2). They state (page 483):

The administration of any depressor substance such as splenic extract, ether, amyl nitrate (?) or magnesium sulphate caused a marked drop in arterial blood pressure followed by a slow rise to normal. Practically coincident with the drop in arterial blood pressure was a sudden rise in cerebral sinus pressure. This usually occurred immediately after the sudden drop, and not with it. The sinus pressure continued to rise as long as the arterial pressure remained at its lower level. As the femoral pressure gradually returned to normal, the sinus pressure slowly dropped.

We do not doubt that such changes may occur—we observed a similar condition a few times (see figs. 3 and 12) but we do not agree that this is the usual change. Out of a total of about ninety injections of tissue extracts having a depressor action, we observed but a few well-marked instances of a condition which Frazier and Peet say is typical. (See table 6, line 8, and figs. 3 and 12). They do not state in how many animals the observations upon which they base their conclusions were made and in none of their tracings do they show a record of the venous changes recorded simultaneously with the arterial and fluid changes. Hence the conclusion must be drawn that their opinion was based on insufficient data, for in our paper we have recorded ninety simultaneous measurements of fluid and venous pressures, and of this number only a very few agree absolutely with what the authors mentioned consider typical.

As further proof for our contention that there is a very close correspondence between venous and fluid pressures, and that these in general follow the changes in the arterial pressures, we would refer the reader to figure 5 and to table 1 which show an average of all the pressure changes produced by kidney extract. It will be observed that for five injections there is an average fall in the general and circle of Willis arterial pressure of 38 and 27 mm. respectively, while in the venous

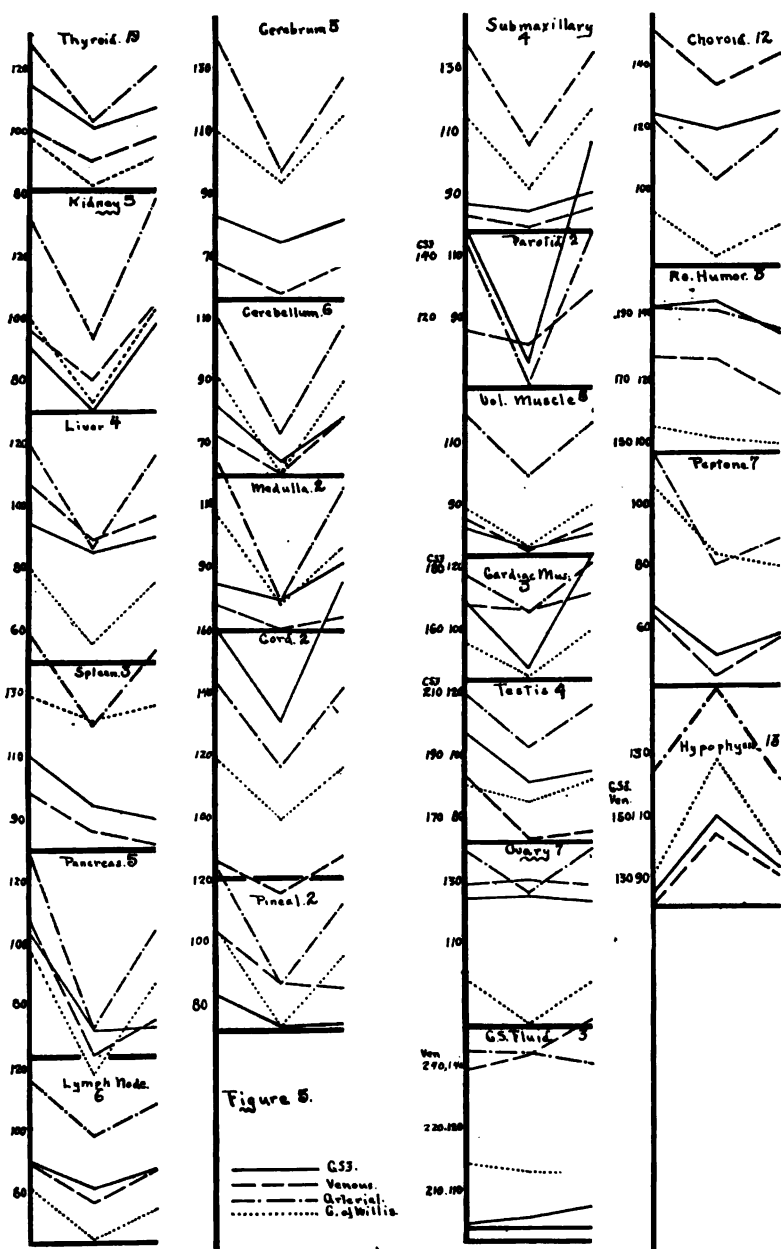


Fig. 5. This figure shows in diagrams the effect on all the pressures of all the tissue extracts. The figures are the averages of the pressures before, during and after the action of the extracts. The number of injections made in each case is indicated by the figure opposite the name of the tissue. Note the marked parallelism between venous and fluid pressures.

and fluid pressures there is an average fall of 10 and 16 mm. respectively. The average for the final readings of pressures shows each of them to be higher than the initial ones were.

If we study in detail the results obtained from extracts with a depressor action as shown in tables 1, 2 and 3, we must observe the following facts: In the case of ovarian extract considering only the average of seven experiments:

Arterial pressure fell 17.1 mm., then rose 18.0 mm.—a gain of 0.9 mm.

Circle of Willis pressure fell 13.9 mm., then rose 13.0 mm.—a loss of 0.9 mm.

Torcular venous pressure fell 18.6 mm., then rose 18.2 mm.—a loss of 0.4 mm.

Fluid pressure fell 19.4 mm., then rose 18.7 mm.—a loss of 0.7 mm.

In the case of testicular extract the averages of four experiments show:

Arterial pressure fell 16.5 mm., then rose 13.5 mm.—a loss of 3 mm.

Circle of Willis pressure fell 5.0 mm., then rose 6.5 mm.—a gain of 1.5 mm.

Torcular venous pressure fell 9.2 mm., then rose 2.0 mm.—a loss of 7.2 mm.

Fluid pressure fell 15.5 mm., then rose 3.75 mm.—a loss of 11.75 mm.

In the case of liver extract the averages of four experiments show:

Arterial pressure fell 33.5 mm., then rose 30 mm.—a loss of 3.5 mm.

Circle of Willis pressure fell 24 mm., then rose 20 mm.—a loss of 4 mm.

Torcular venous pressure fell 17.25 mm., then rose 7.75 mm.—a loss of 9.5 mm.

Fluid pressure fell 9.25 mm., then rose 5.5 mm.—a loss of 3.75 mm.

In the case of extract of the spleen the averages of three experiments show:

Arterial pressure fell 29.33 mm., then rose 24 mm.—a loss of 5.3 mm.

Circle of Willis pressure fell 7.5 mm., then rose 5 mm.—a loss of 2.5 mm.

Torcular venous pressure fell 9.3 mm., then fell 4.7 mm.—a loss of 14.0 mm.

Fluid pressure fell 14.7 mm., then fell 4.0 mm.—a loss of 18.7 mm.

In the case of extract of the pancreas the averages of five experiments show:

Arterial pressure fell 55.6 mm., then rose 31.6 mm.—a loss of 24 mm.

Circle of Willis pressure fell 40.45 mm., then rose 29.2 mm.—a loss of 11.25 mm.



Torcular venous pressure fell 42.8 mm., then rose 11.2 mm.—a loss of 31.6 mm.

Fluid pressure fell 30.8 mm., then rose 2.2 mm.—a loss of 28.6 mm.

In the case of submaxillary extract the averages of four experiments show:

Arterial pressure fell 41.0 mm., then rose 38.5 mm.—a loss of 2.5 mm.

Circle of Willis pressure fell 23.0 mm., then rose 25.3 mm.—a gain of 2.3 mm.

Torcular venous pressure fell 3.0 mm., then rose 5.75 mm.—a gain of 2.75 mm.

Fluid pressure fell 2.5 mm., then rose 5.25 mm.—a gain of 2.75 mm.

In the case of the parotid gland extract the averages of two experiments show:

Arterial pressure fell 44.5 mm., then rose 48.0 mm.—a gain of 3.5 mm.

Torcular venous pressure fell 14.5 mm., then rose 27 mm.—a gain of 12.5 mm.

Fluid pressure fell 42 mm., then rose 69 mm.—a gain of 27 mm.

In the case of lymph gland extract the averages of six experiments show:

Arterial pressure fell 17.8 mm., then rose 11.8 mm.—a loss of 6 mm.

Circle of Willis pressure fell 15.75 mm., then rose 9.25 mm.—a loss of 6.5 mm.

Torcular venous pressure fell 12.3 mm., then rose 10.1 mm.—a loss of 2.2 mm.

Fluid pressure fell 8.5 mm., then rose 5.9 mm.—a loss of 2.6 mm.

In the case of skeletal muscle the averages of five experiments show:

Arterial pressure fell 17.4 mm., then rose 35.2 mm.—a gain of 17.8 mm.

Circle of Willis pressure fell 12.3 mm., then rose 13.5 mm.—a gain of 1.2 mm.

Torcular venous pressure fell 10.2 mm., then rose 8.6 mm.—a loss of 1.6 mm.

Fluid pressure fell 6.8 mm., then rose 4.8 mm.—a loss of 2 mm.

In the case of cardiac muscle the averages of three experiments show:

Arterial pressure fell 16.5 mm., then rose 16.75 mm.—a gain of 0.25 mm.

Circle of Willis pressure fell 11.8 mm., then rose 14.3 mm.—a gain of 2.5 mm.

Torcular venous pressure fell 6.9 mm., then rose 7.8 mm.—a gain of 0.9 mm.

Fluid pressure fell 12.3 mm., then rose 16.3 mm.—a gain of 4 mm.

In the case of peptone solution the averages of seven experiments show:

Arterial pressure fell 36.2 mm., then rose 8.2 mm.—a loss of 28 mm.

Circle of Willis pressure fell 22.0 mm., then fell 4.0 mm.—a loss of 26 mm.

Torcular venous pressure fell 17.4 mm., then rose 10.8 mm.—a loss of 6.6 mm.

Fluid pressure fell 15.8 mm., then rose 6.8 mm.—a loss of 9.0 mm.

A study of the results of tables 1, 2 and 3 as just completed brings out convincingly the fact that in every case where there was a decrease in venous pressure there was at the same time a decrease in fluid pressure; when there was an increase in venous pressure there was at the same time an increase in fluid pressure. These pressures always vary in the same direction. If after the fall the venous pressure rose above the original level, as was the case in extracts of the kidney, submaxillary gland and parotid gland, and cardiac muscle, the level of the fluid rose above the original level; if after the fall the venous pressure remained below the original level during the period of observation, as was the case following the injection of extracts of ovary, testicle, liver, spleen, pancreas, lymph gland, skeletal muscle and peptone, the fluid remained below the original level. This parallelism certainly is more than a coincidence. Further, in one case, the spleen, the arterial pressure fell and then rose, but the venous pressure instead of rising as is ordinarily the case after depressor extracts continued to fall although the arterial pressure was rising. It will be noted that the fluid pressure continued to fall with the venous pressure rather than rising with the arterial pressure. Since it has already been shown that changing venous pressure by a moderate amount changes fluid pressure in the same direction, and to some degree proportionally, but changing the fluid pressure by less than 100 mm. of salt solution does not alter the venous pressure, it is evident that the determining factor for the fluid must be venous pressure and therefore the fluid pressure changes because of the changes in venous pressure. All the changes observed here can be explained readily on a mechanical basis and it is not necessary to invoke a theoretical increased or decreased activity of the mechanism producing the fluid in order to explain all the phenomena observed. The extracts when given intravenously produce certain changes in the venous pressure, and these changes in venous pressure determine the behavior of the fluid. This gives a purely mechanical explanation for the behavior of the fluid following the injection of tissue extracts. This is the conclu-

sion already reached by Frazier and Peet (2), although, as has been pointed out, their ideas regarding the behavior of the venous pressure are the opposite of those actually observed by us in practically every one of ninety injections of extracts of various organs. Experiments on other parts of the field (6) show that the behavior of venous pressure is the same no matter whether the manometer or the outflow method of studying the behavior of the fluid is employed.

This relationship between all the pressures, as shown by the averages of all of the injections of any one extract, serves to show what will be observed following injection of any depressor extract—namely, that the changes produced in the fluid are mechanical, and that they are dependent primarily on venous changes. Allowing for the error in the method employed, all the changes observed in the fluid following the injection of depressor extracts can be accounted for by the changes in the venous pressure. The venous pressure is, it is true, ultimately dependent upon the arterial pressure, but can and does undergo variations independent of arterial pressure, and while it has been shown that arterial pressure influences fluid pressure the chief influence is still by way of the venous pressure. Our series of experiments gives abundant evidence that arterial and venous changes do not necessarily run parallel (6), particularly where the changes are comparatively small. Therefore it will be seen that for any adequate interpretation of the changes taking place in the fluid pressure, it is absolutely necessary to know what changes are taking place simultaneously in the venous sinuses of the skull. General venous pressure elsewhere in the body has no bearing on this question, as was shown in an earlier paper.

The lymphagogues are in the same class as the extracts already discussed. The effect produced by them on the fluid pressure was in every way comparable to the changes produced by the tissue extracts, insofar as they produce any vascular effects. Peptone caused a decided fall in arterial pressure and consequently marked changes in the fluid pressure. Sugar caused little vascular change and therefore little change in the fluid pressure, showing that whatever the change, the result was purely mechanical.

How, then, if arterial and venous pressures both fall following the injection of most tissue extracts instead of the former falling and the latter rising as is stated by Frazier and Peet, can we explain the mechanism of increased outflow of fluid following the injection of these extracts? That the intravenous injection of these extracts produces an increased outflow of fluid at least temporarily is not to be doubted. In-

creased venous pressure, if such could be shown, would furnish the *vis a tergo*, but we have shown that venous pressure as well as fluid pressure falls instead of rising, so some other explanation must be sought. The changes recorded by Frazier and Peet are much greater than we secured with the animal on his side. As was pointed out, the position they used favored the escape of preformed fluid by making the point of drainage the lowest point in the system. We believe that using the outflow method, tissue extracts produce their effect by reducing arterial and venous pressure, thus shrinking the sinuses and permitting the fluid in the cerebral cavity, which up to this time has been prevented from passing the strait, at the region of the mid-brain, to pass into the region of the fourth ventricle and out through the needle. If this is true then dogs with no fluid over the cerebrum will give negative results. Any worker will testify that many animals fail to show an increase. One further possibility has been pointed out: If the resistance offered by the fluid in passing through the needle is less than that offered by the venous blood in passing by natural channels (veins) to the heart, then the amount of blood accumulating in the sinuses would increase at the expense of the fluid. The accumulation of blood would thus displace an equal amount of fluid, thus setting up a pseudo-secretion without a rise, or perhaps even with a fall of venous and fluid pressures. The conditions present in Frazier and Peet's experiments are almost ideal for this mechanism of outflow for the fluid has only to flow under the influence of gravity down the plane offered by the nervous system and meninges to escape. The graduated tube adjusted level offers some resistance but very little in the outward direction, and this little is easily overcome by the influence of gravity.

We are convinced that the effect of tissue extract upon fluid outflow is simply to facilitate the escape of preformed fluid, either by making the transit of fluid past the various straits easier or by producing a condition whereby venous blood accumulates in the skull at the expense of the fluid. This is the conclusion reached by Frazier and Peet in their study of the tissue extracts thus far reported, but our conception of the mechanism involved is different from theirs, theirs being founded upon the erroneous idea that venous pressure rises in the skull just after the fall in arterial pressure, whereas our work shows that venous pressure falls along with arterial in almost every case.

## EXTRACTS HAVING A PRESSOR EFFECT

Extracts from the adrenal and from the posterior lobe of the hypophysis produced a pressor effect on the vascular system. Since hypophysis extract is claimed to have a specific action on the choroid plexus, (3) it will be discussed under the third group. Only a few extracts were made from the adrenals because the effect produced by the fresh extract was entirely comparable with that of the commercial preparation, but less active. Hence for additional data we refer the reader to the tables in another article (6), which give the changes produced by adrenalin chloride. The rise in arterial pressure following injection was accompanied by a corresponding rise in venous pressure and cerebrospinal fluid pressure, the fall in arterial pressure by parallel decline in venous and fluid pressures. The changes in the fluid pressure with adrenal extract are so obviously dependent on the vascular changes that workers are agreed that the changes are mechanical. Since the arterial changes, particularly after atropine, are usually very pronounced and continue for a relatively short time, a closer relationship is to be seen between arterial and fluid changes because the venous pressure follows the arterial more closely. Therefore, even though previous workers did not record venous pressure, they nevertheless arrived at the correct conclusion.

## EXTRACTS SAID TO HAVE A SPECIFIC ACTION ON THE RATE OF FORMATION OF THE CEREBROSPINAL FLUID

*Pressor extracts*

*Extracts of the pituitary and the adrenals.* In accordance with the principles laid down in the earlier work, it is held that before it is necessary to ascribe an increased fluid pressure or an increased fluid outflow to an increased rate of formation of fluid, all of the physical factors capable of producing such change must be measured and shown to be unchanged, or altered in such manner as to produce a change opposite to that noted in the fluid. It has already been shown that alterations in the venous pressure invariably produce a change in the fluid pressure in the same direction and to some degree proportionally. Arterial pressure modifies the fluid pressure in some cases, but does not modify it in others, the determining factor being the amount of fluid in the canal: with a large amount of fluid in the canal the fluid pressure rises or falls with the arterial, with a small amount of fluid in the canal

the arterial pressure loses its effect because the dilatation and constriction in the artery are not sufficient to affect the fluid pressure, for all the force is carried by the arterial wall without much if any dilatation, and so the force is not transmitted to the fluid. The question of the change of pressure in the artery would not enter to any degree, if at all, in the outflow method, for most of the excess of fluid has escaped before the experiment is begun. It seems highly improbable that the alteration in the volume in the arterial side of the capillary due to increased arterial pressure will to any degree equal the amount of change produced by the dilatation of the vein produced by increased accumulation of blood in the sinuses by increased venous pressure. The influence of respiration has already been adequately discussed. It was shown to be variable, both as regards amount and character of change. Sometimes there is an increased, sometimes a decreased outflow, depending upon whether there was free or difficult communication between the various cavities, and as to whether there was much or little fluid stored in one of the cavities shut away from the *cisterna magna* by the position of the brain and medulla. Thus it becomes clear that it is absolutely necessary that venous and arterial pressure and the influence of respiration be measured in every case before the significance of the rise in fluid pressure, or the increase in the outflow can be properly estimated.

Applying this knowledge to the effects of the injection of extracts of the pituitary and of the adrenal upon the formation of fluid, it is necessary that a careful study of the behavior of all these pressures be made in order to ascertain whether anything more than pure mechanical factors are involved in the rise in the fluid pressure and the increased outflow of fluid from the canal after the injection of pituitary extract or adrenalin.

A survey of the recent literature shows that there are divergent opinions regarding the influence of extracts of the pituitary on the formation of the fluid. Weed and Cushing (3) conclude: "Extracts of the posterior lobe of the hypophysis increase the rate of production of cerebrospinal fluid (choroidorrhea) by stimulating the secretory activity of the choroid plexus." Dixon and Halliburton classify pituitary extract with substances producing "no increase or diminution" in outflow (p. 239). Later (5) they agree with Weed and Cushing that in animals breathing naturally, pituitary extracts produce an increased outflow of fluid, but assert that if the animal is deeply anesthetized and ventilated artificially, pituitary extracts produce no increased outflow. They ascribe the increased outflow seen in the normally breathing animal to asphyxia because of bronchial constriction.

Regarding the influence of adrenalin, Dixon and Halliburton state: "A sudden rise in arterial pressure such as is produced by adrenalin in dogs with cut vagi, will produce a mechanical pressing out of accumulated fluid." Earlier observers, particularly Spina (7), were of the opinion that adrenalin increased the fluid outflow by increasing the transudation through the pial vessels.

It is thus evident that there is a divergence of opinion in regard to the action of these drugs. We do not know whether or not there is an increase in the amount of fluid formed; or if there is an increase, whether the increase is due to accelerated secretion due to stimulation or increased transudation. If there is increased stimulation of the secretory mechanism, we do not know from the literature whether there is an active principle found in the pituitary stimulating the cells specifically or whether the extract of pituitary produces its effect indirectly by asphyxia. Granting for the time being the unification of views suggested by Dixon and Halliburton for the differences between their findings and those of Weed and Cushing, and thus granting that in the naturally breathing animal there is an increased formation of fluid as manifested by increased outflow by the outflow method, or increased pressure by the manometer method, an exhaustive study of the behavior of arterial, venous and fluid pressures must be made in order to rule out the possibility of the change being purely mechanical.

Regarding adrenalin Dixon and Halliburton say: "A sudden rise of arterial pressure, such as is produced in dogs with cut vagi, will produce a mechanical pressing out of accumulated fluid" (p. 239), a finding with which we agree absolutely.

Since pituitary and adrenal extracts both contain an arterial pressor substance, and since changes in arterial pressure produce so frequently changes in other parts of the vascular system, particularly in the venous circulation in the skull, which has already been shown markedly to influence the behavior of the fluid, the question naturally arises as to whether the assumption that pituitary extracts have a stimulating effect, while the adrenalin has only a mechanical effect upon the fluid, is logical.

Of course it is well known that there are some marked differences between the actions of adrenalin and extracts of the pituitary: Adrenalin has been shown (8) to stimulate the secretion of the salivary and lachrymal glands supposedly with increased circulation in the former (9) although secretion in most glands is depressed by the powerful vasoconstriction (10). Pituitary extracts are said to depress the secretion

of the submaxillary partly by vasoconstriction, partly by direct depression of the salivary cells (9). Thus it is clear according to the literature that adrenalin stimulates, extracts of the pituitary depress secretion of the salivary glands, exactly the opposite of the findings in the case of the cells forming the cerebrospinal fluid, where adrenalin has no effect and pituitary extract stimulates them to greater activity.

There is almost as much difference between the effects of these drugs upon blood pressure as upon salivary secretion: Adrenalin intravenously produces a rapid rise in the blood pressure which begins in three to ten seconds, reaches a maximum five to ten seconds later, and disappears in twenty seconds to two minutes. Except for the latent period all these phases are modified by the dose employed and by the action of the vagi. Pituitary extracts frequently produce a fall, and then a slow, long-continued rise. The former is not constant and may be due to some extraneous material in the preparation. The rise begins in less than a minute and lasts five to thirty minutes. Secondary rises after the first are not uncommon. The point of action as well as the character of the curves is different: both drugs act peripherally. Adrenalin is believed to act on the physiological sympathetic nerve endings. The physiological endings must be affected because the action of adrenalin is prevented or reversed by the previous action of apocodeine (11) and ergotoxine (12). If the point of action were the muscle, the reaction would be the same both before and after these drugs. Further Maass (13) has shown that the accelerator (sympathetic) nerves to the heart carry vasodilator fibers to the coronary arteries. Langendorff (14) has shown that the effect of adrenalin on the coronary area is dilatation, not constriction. These two facts are further support for the idea that adrenalin acts on the sympathetic endings and not on the muscular wall, for we have no reason to believe that the muscles of the coronary vessels differ essentially from those of other vessels. That the action of adrenalin does not depend upon the anatomical nerve endings is shown by the fact that the drug has a typical effect upon a part when the nerve endings have degenerated after section of the nerve (15). Pituitary extract acts upon the muscle of the wall for its effect is typical after apocodeine and ergotoxine. In addition to the points already discussed there is another marked difference between adrenal and pituitary extracts in their effects upon the blood pressure: Adrenalin not only produces its effect repeatedly, but almost quantitatively (16). Pituitary extracts produce practically no effect on a second injection immediately after the blood pressure has returned to normal after a



first injection. Some claim there may even be a reversal of the curve on repeated injections (17), others claim that the effect may be reproduced if the doses employed are small and the injection is not repeated until about a half or three-quarters of an hour has elapsed (18). Thus there are enough differences between the action of adrenalin and pituitary extract to warrant the belief that one might stimulate some cells to increased secretion, the other might produce increased outflow by purely mechanical means.

Under the belief that the truth regarding all the factors can be arrived at only by the study of rather a large number of cases, we undertook the work on a number of dogs. Using the manometric method, twelve injections of fresh extracts of dog pituitary were made into eleven dogs. Five were extracts of the entire organ, three were extracts of the anterior, four of the posterior lobes, four injections were made with proprietary preparations from various drug houses (table 4).

In experiment 1 the extract of the entire hypophysis of one dog was injected. There was first a pressor effect, and there were simultaneous rises in venous and fluid pressures. In experiment 2 a similar injection gave first a slight depressor, but later a pressor effect on arterial pressure. This rise may have been due in part to asphyxia, for the animal ceased breathing for a time. And here as before both venous and fluid pressures rose simultaneously. In experiment 3 the injection of 5 cc. of the extract gave an immediate pressor arterial effect followed by a long-continued high pressure. The rises in fluid and venous pressures were again simultaneous and practically equal in amount. In all the experiments described the final pressure readings were all higher than the normal preceding the injection. In experiment 4 the injection of 5 cc. of extract of hypophysis produced a depressor arterial effect accompanied by a rise in venous and fluid pressures, here the final reading for arterial pressure was lower than the initial one, but the venous and fluid pressures were higher. It is well to note again at this point, as was done in the earlier paper, that arterial and venous pressures do not always run parallel. This is true after the injection of pituitary as well as for other extracts. This experiment is very similar to the results with choroid plexus extract in experiment 8 in which the high point of fluid and venous pressures came with the low point in arterial pressure (see fig. 12), and agrees with what Frazier and Peet believe to be the usual result with depressor extracts. A second injection of hypophysis extract in this same animal (exper. 5) gave practically opposite results. There was a slight pressor effect on arterial pressure accompanied by a

slight fall in venous and fluid pressures. The final readings in arterial pressure were slightly less than the initial pressure, while the venous and fluid pressures were practically the same. The variability shown in the two injections of the same extract, into the same animal, emphasizes again the importance of taking the venous pressure simultaneously with

TABLE 4  
*Extract of hypophysis*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
Entire hypophysis												
1	162	183	165	120	132	127	145	159	160	162	168	170
2	134	132	150				77	90	95	109	110	130
3	132	160	146	100	136	124	83	112	109	85	108	107
4	140	124	126				90	117	101	105	118	111
5	150	156	140				75	72	77	88	83	87
Posterior lobe extracts												
1	146	172	150	84	144	120	276	356	304	257	321	274
2	84	106	78	62	80	62	253	239	230	138	187	142
3	112	194	146	82	164	112	89	149	140	87	143	140
4	150	186	90	114	152	72	129	81	84	142	98	114
Commercial extracts of pituitary												
1	106	160	116				107	122	110	69	70	66
2	88	106	16				96	97	98	98	127	90
3	124	128	124	100	104	100	108	129	110	106	129	109
4	108	158	110	78	106	66	59	149	85	193	285	192
Average	125.8	151.1	119.7	92.5	127.2	97.9	122.1	144.0	131.0	126.0	149.7	133.2
Anterior lobe extracts												
1	152	154	156	118	119	119	309	315	321	282	288	296
2	124	118	128	92	94	104	111	103	91	153	148	138
3	72	82	88	54	54	66	241	270	262	124	140	105

the other pressures, for without knowing the changes taking place in the venous sinuses, the explanation of the changes in the fluid can be guess-work only.

*Anterior lobe extracts.* Extracts of anterior lobe were used in three experiments: Little vascular change was produced by these extracts

and the changes in arterial, venous and fluid pressures were all parallel. These changes in pressure were typical and are to be explained on a purely mechanical basis.

*Posterior lobe extract.* In experiment 12 the injection of 2 cc. posterior lobe extract gave immediate and long-continued pressor effect (see fig. 6). The venous and fluid pressures rose simultaneously with the arterial and the high point in all of them was reached at practically the same time. The fall in all the pressures was gradual but in every case the final readings were slightly higher than the initial ones. A study of the tracing will show the close relationship of all the pressures and particularly of the venous and fluid pressures. The comparatively greater rise in circle of Willis pressure as compared to general arterial pressure following the injection of posterior lobe extract is a striking feature of this tracing. This effect appears to be quite common after pituitary injections, but its significance is not understood. In experiment 2 the injection of 2 cc. posterior lobe extract gave an immediate though comparatively short pressor effect. There was a corresponding rise in fluid but little rise in venous pressure. The final reading showed the fluid pressure to be comparatively higher than either the venous or arterial. This might be interpreted as an actual increase in fluid due to secretion but we do not believe it is: this increase in fluid is the exception rather than the rule. It should appear more frequently than once in fourteen injections; further the same change is observed in the case of other extracts. See line 9 in table 7 showing the effect of thyroid injections. The increase is very small in amount (1 cm. on the manometer equals 0.03 cc.) and therefore after allowing for error in the method, the increase is negligible. Further we know that a rise in arterial pressure can produce a rise in fluid pressure.

In experiment 3 the injection of 4 cc. posterior lobe extract gave an immediate short pressor effect, followed by a long pressor effect. The high points were reached in the venous and fluid pressures before those in the arterial pressures. There was an immediate gradual parallel decline in venous and fluid pressures in spite of a decided second pressor arterial effect. The final arterial pressures were lower while the venous and fluid pressures were practically the same as at the first readings. This injection shows clearly the variations that are so often seen between arterial and venous pressures but at the same time it gives additional support to our contention that there is a close correspondence between venous and fluid pressures.

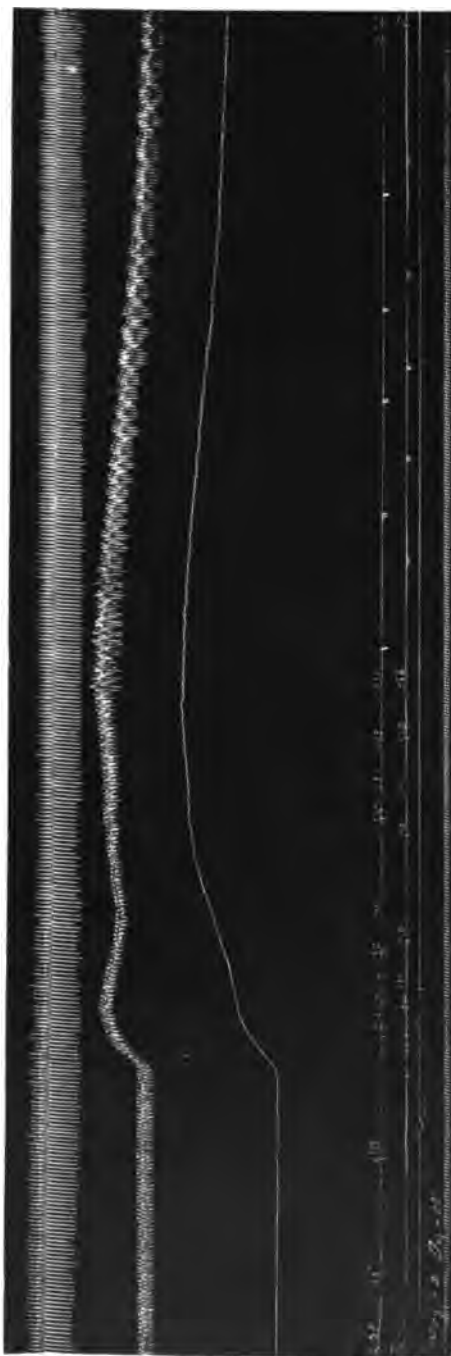


Fig. 6. This figure shows the effect of the injection of an extract of posterior lobe of the hypophysis. The pressure method of recording was employed. Note that all pressures had returned almost to normal by the time the tracing was discontinued. Complete readjustment was noted later.

Pituitary Liquid (Armour) was injected into a number of animals but the vascular changes produced were quite variable. In experiment 4 a marked and rather long-continued pressor effect was produced; the changes in the venous and fluid pressures were in the same direction as the arterial but comparatively smaller. Here the final pressures, with the exception of the fluid pressure, stood higher than the initial ones. This is in striking contrast to the observation in experiment 3 in which the fluid pressure was comparatively higher than the other pressures—if the one were called secretion, then the other with equal right could be called absorption.

A careful survey of the effects produced by the injection of pituitary extracts shows that although the pressor changes were of longer duration than those produced by adrenalin, nevertheless the fluid changes followed the vascular changes and in particular the venous changes, closely. With the single exception, noted above, in experiment 2 (posterior lobe extract) there was no evidence of a "choroidorrhea" such as is described by Weed and Cushing (3). In fact all of the changes observed can be accounted for by vascular changes, the most important of which are the venous pressure changes. That Weed and Cushing recognize the possibility of the fluid being affected by the venous pressure is shown by their own statement: "in view, therefore, of the possible influence of these physical factors on the discharge of the fluid, it is hazardous to assume that certain agents stimulate the secretion of the choroid plexus, merely because of an increased flow from a cannula." Had they measured venous pressure simultaneously with the arterial and respiratory changes, they would undoubtedly have observed one more of the physical factors concerned in the outflow of the fluid. They recorded the respiration but evidently did not regard it as significant—that it may be an important physical factor where the outflow method is employed has been clearly shown in a previous article (6). The amount of fluid in the subarachnoid spaces at the time of the injection and the limitation of the fluid pressure to the resistance of the cannula employed are other physical factors which they failed to consider at all.

The basis for their conclusion that the extract of the posterior lobe of the hypophysis produces a "choroidorrhea" is briefly stated as follows: *a*, that there was an actual secretion because there was no recession of fluid in the cannula; *b*, that the catheter was in the ventricle rather than the subarachnoid cistern; and *c*, the apparent fatigability of the response to repeated injections. The weakness of their first

and second arguments has already been pointed out clearly (6). Briefly they are as follows: *a*, the subarachnoid spaces are surrounded on the one hand by the undilatable calvarium and on the other by the relatively incompressible brain substance; hence little negative pressure could be developed to cause a recession of the fluid into the catheter or tube. Therefore, a lack of recession of fluid would mean nothing. *b*, The presence of the catheter in the ventricle does not eliminate the possibility of a transudation of the fluid from the capillaries, which under the experimental conditions might be greatly increased; neither does it eliminate the possibility of a backward flow through the aqueduct of Sylvius, unless the catheter is placed into the aqueduct. The weakness of their third argument lies in the fact previously stated, i.e., that a second injection of pituitrin produces little or no vascular effect. If the changes produced in the cerebrospinal fluid by the injection of pituitrin are mechanical, as we believe they are, then if a second injection produced little vascular change, a correspondingly small fluid change would be expected. Furthermore, if the preformed fluid were forced out to a considerable degree following the first injection, there would be little left to be forced out following a second injection. That the amount of fluid in the subarachnoid spaces is important in determining the outflow from the cannula has likewise been clearly shown in the previous paper. It would appear, therefore, that it is entirely unnecessary to assume that the cessation of flow is due to the fatigability of the choroid because this (cessation of outflow) can be satisfactorily explained by the physical factors involved.

The tracing presented by Weed and Cushing to prove that the outflow is independent of vascular changes is not complete. To compare with records I and II of their paper we show figure 7, a graph which reproduces almost exactly the arterial curve which they show. As can be seen from the readings of fluid and venous pressures on our curves, synchronous with the fall in arterial pressure there was a rise in venous pressure, a rise reaching a maximum of 28 mm. above normal. Almost simultaneously with the minimum of arterial pressure and synchronous with the rise in venous pressure was a rise in fluid pressure of 23 mm. Both venous and fluid pressures fell below normal as arterial pressure rose, and then rose to normal as arterial pressure reached its maximum. This rise in fluid and venous pressure came at about the time when there is in records I and II an increase in fluid outflow, and a rise in venous pressure is able to produce just such an outflow as is shown by the ligations of the jugulars shown in the preceding paper. We are con-

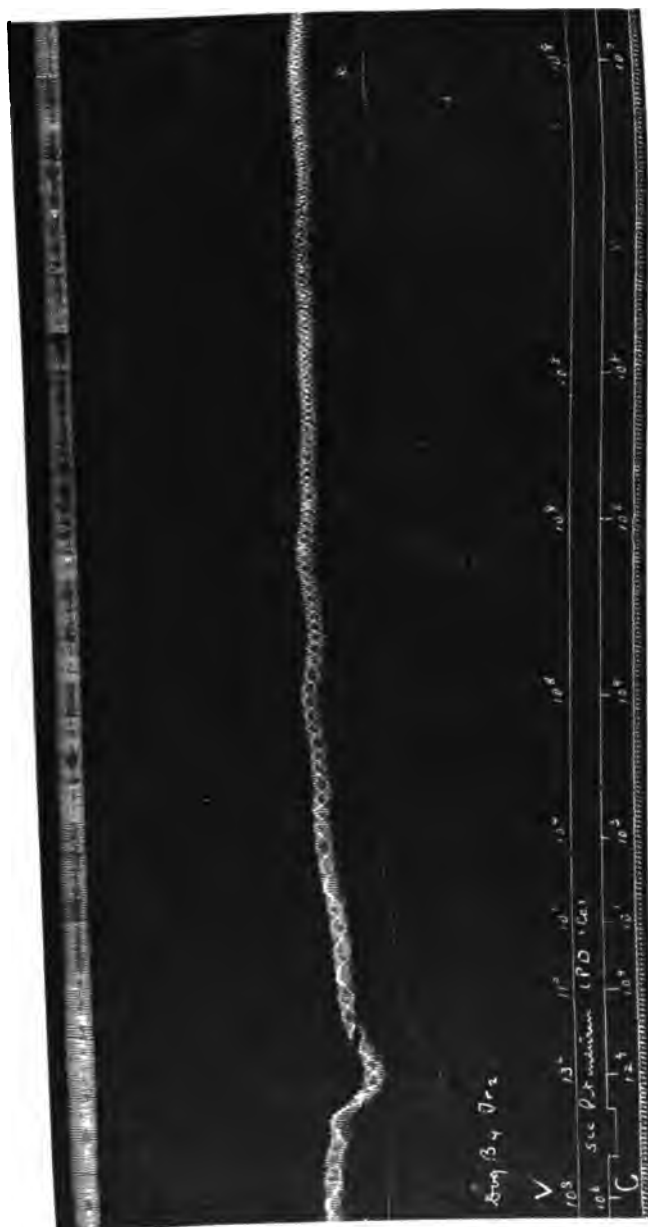


Fig. 7. This figure shows that with the preliminary fall in arterial pressure common soon after pituitrin injections there is frequently seen a marked rise in venous pressure with a corresponding rise in fluid pressure.

vinced that if the authors mentioned had had a venous tracing of the experiments shown in I and II they would have found a rise in venous pressure synchronous with their increased outflow. Figure 7 shows such a venous pressure rise with a simultaneous fluid pressure rise. Figure 8 shows a similar result where the outflow method was employed, a fall and then a rise in arterial pressure with a rise and then a fall in venous pressure with an increased outflow of fluid during the venous pressure rise (see fig. 8).

To compare with record III we show figure 6. This curve reproduces almost exactly their arterial curve. As can be seen, their increased outflow of fluid came while the arterial pressure was rising, dropped ap-

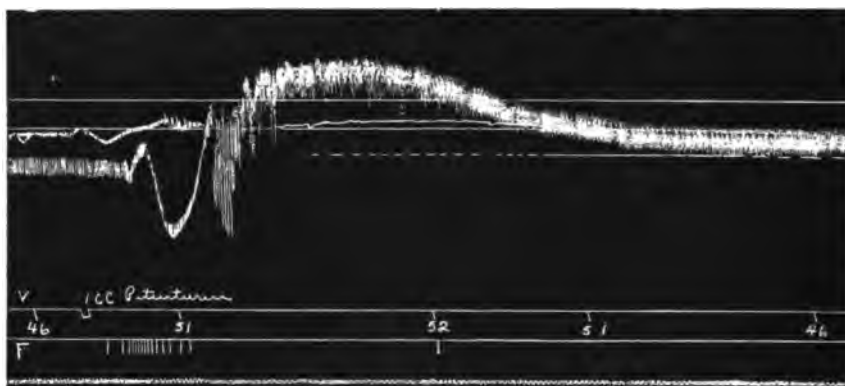


Fig. 8. This figure shows that with the preliminary fall in arterial pressure common soon after pituitrin injections there is frequently seen a marked rise in venous pressure; with this rise in venous pressure comes an increased outflow of fluid.

proximately to the normal rate when the highest point in the curve was reached, and practically ceased at a time when the arterial pressure was still far above normal. Our curve shows that at the time when their maximum outflow came venous and fluid pressures were rising synchronously; the former rose from 276 mm. to 356 mm., a rise of 80 mm.; the latter rose from 257 mm. to 324 mm., a rise of 67 mm. It is therefore self-evident that the cause of the increased outflow was not necessarily an increase in the amount of fluid in the skull, due to a choroidorrhea, it may have been—probably was—simply the expulsion of fluid by the increased venous pressure produced by the action of the drug. The cessation of flow with arterial pressure still high shown in records III



and IV means simply that enough fluid had been expelled to accommodate the venous blood accumulated in the sinuses, and further expulsion ceased. The failure of recession of the fluid finds a number of explanations: no negative pressure can be developed because of a lack of positive pressure in expelling the fluid, the high venous pressure may cause increased transudation, or what seems more plausible, the deficit in the vicinity of the needle is made good by fluid under positive pressure in other regions of the canal temporarily shut off from the needle by the position of the central nervous system. In actual fact we have seen the fluid recede in the cannula in several experiments, a fact which may

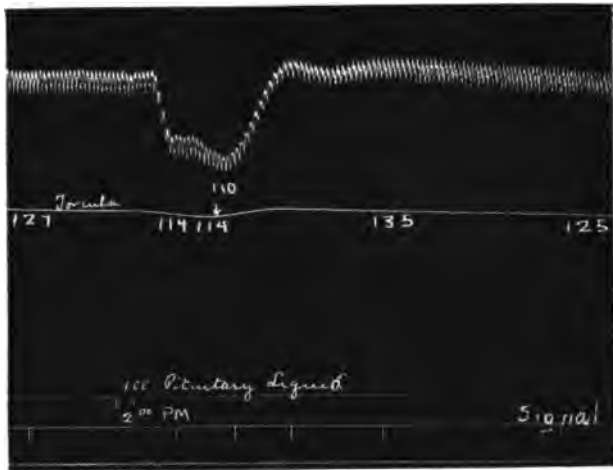
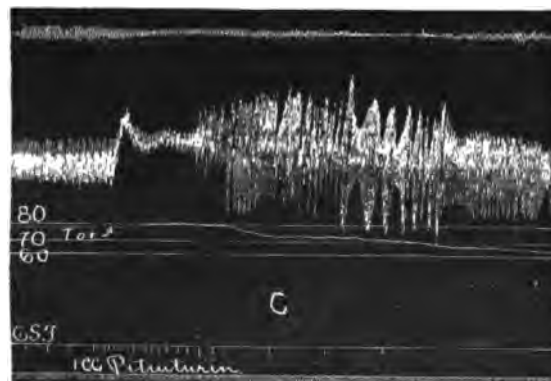
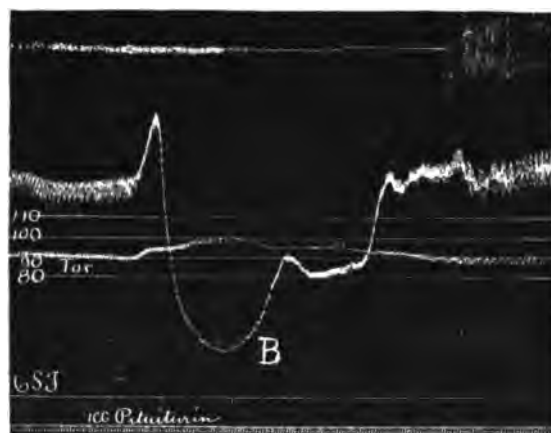
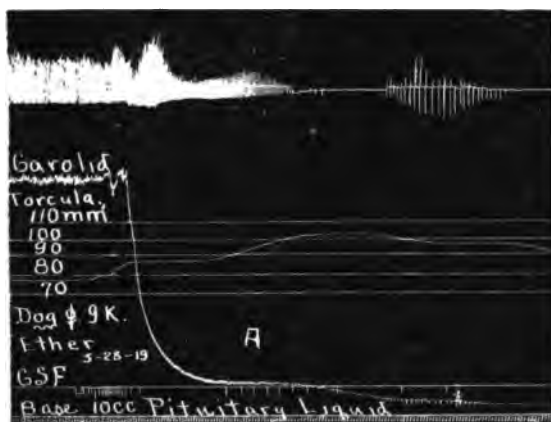


Fig. 9. This figure shows that after pituitrin the venous pressure may fall with the fall in arterial pressure.

point to the latter as the more likely of the three explanations offered. The differences in results in their tracings V and VI are due to differences in the behavior of the venous pressure in the two injections. We believe record V showed no effect on fluid outflow because there was no rise in venous pressure. We believe record VI shows the marked increase in fluid outflow because of a marked rise in venous pressure after the injection. As we pointed out before, the arterial pressure is no criterion of what is taking place in venous pressure, and while we have no basis for the assumption that venous pressure rose in record VI and did not rise in record V, we believe such is the case from our experience in similar conditions of experimentation. Weed and Cushing cannot



tell what happened in the vein during their experiments for the reason that they did not measure that pressure. To prove our point we cite tracings 8 and 9 where a fall in arterial pressure after pituitrin was in one case accompanied by a rise, in another case by a fall in venous pressure.

To compare with their record VII we show figure 10 *A*, an experiment in which following a large dose of pituitrin there was a marked fall in arterial pressure with increased fluid outflow. The remarkable fact brought out in our tracing is the fact that not only did the venous pressure rise from the first, but that it rose in two stages and each stage of increasing venous pressure was accompanied by an increased outflow of fluid, an increased outflow which was followed by a cessation each time venous pressure became stationary. It is therefore obvious that the factor which determines fluid outflow is not the actual venous pressure level, but the question as to whether the pressure is rising and the blood is accumulating in the sinuses. The venous pressure following pituitrin injections is not determined by the arterial pressure in the carotid, hence the mere fact that arterial pressure fell does not prove that venous pressure fell with it and hence the increased secretion of fluid is not the only cause of increased outflow. This increased outflow of fluid is just as satisfactorily explained by an increase in venous pressure or increased accumulation of venous blood as by increased secretory activity of the choroid. In fact there is no reason for invoking the latter at all, for all the facts are adequately explained by the former. Figure 10 *B* shows that pituitrin sometimes decreases fluid outflow, although venous pressure was rising slightly. Figure 10 *C* shows the optimum condition for outflow, increased arterial and venous pressures.

If further evidence were needed to show the dependence of the fluid on the vascular changes produced by the injection of extracts of the hypophysis, a study of all the changes shown in figure 5 should be convincing. The averages show a decided rise in all the pressures following the injection, and while the general arterial pressure returns to a lower

Fig. 10. This figure shows the different reactions of different dogs to the intravenous injection of commercial extracts of the hypophysis. Note in *A* a rapid outflow of fluid with a falling arterial pressure, also that the increased outflow is synchronous with the rises in venous pressure which occurred in two distinct stages. Note in *B* a similar fall in arterial pressure with only a slight rise in venous and a marked decrease in the rate of outflow. Note in *C* a rise in both arterial and venous pressures with a marked increase in the rate of outflow of fluid. These tracings show the importance of the venous pressure in the determination of the mechanism of fluid outflow.

level than at the beginning, all the other pressures remained at a higher level. The increase in the circle of Willis pressure, following the injection of hypophysis extract, an effect already referred to, would undoubtedly be sufficient to account for the high venous and fluid pressures. The point of particular importance, however, is the average change in the fluid; this is practically parallel with the average of the venous changes and if there were a decided increase in the fluid as is claimed, this would not be the case for if there had been a marked increase in fluid, the rise in fluid pressure would have been greater than that of venous pressure. By the manometer method, therefore, there is absolutely no evidence for any increase in the fluid and we believe that if Weed and Cushing had considered all the physical factors concerned in the outflow of the fluid, it would have been unnecessary for them to postulate a "choroidorrhea" to account for the changes they observed.

The increased flow of fluid following the injection of extract of pituitary body is due to the increase in the vascular pressure in the skull. The assumption that there is an increase in the amount of fluid is not necessary to explain the facts.

Frazier and Peet (2) conclude that brain extract has a specific effect because the rate for a given period following injection is greater than normal—in other words, more fluid escapes in a given period following the injection of brain extract than would have escaped in the same time with a normal rate of flow. This increase, they say, is independent of the fall in arterial pressure. We do not doubt that they observed an increased outflow of fluid, we ourselves have noted the same increase under similar conditions but with us, as can be seen from the table, there is a decrease in venous and fluid pressures in every case, just as there was in the case of the other depressor extracts, and not an increase in venous pressure as we have already pointed out Frazier and Peet believe to have occurred at the time the arterial minimum occurred. We are convinced that the increased fluid flow was due to the escape of preformed fluid lying in the adjacent cavities of the subdural space, an escape made possible by the marked shrinkage of the intracranial structures produced by the reduced arterial and venous pressures which permitted the fluid to escape past the natural obstructions and thus to appear at the most dependent portion of the canal, the *cisterna magna*, and escape through the needle. With the rise in arterial and venous pressures the intracranial structures again expand and the passage way is again obstructed, and a cessation of flow follows. Why nervous tis-

sue produces so much more marked an increase is obviously due to the more marked fall in arterial and venous pressures, and therefore to the greater shrinkage of structures and the freer transit of fluid through the straits. The above explanation makes it obvious that falls in arterial and venous pressures will not always produce an increased flow. With the dog on his side, the results are positive only in those cases where there is an accumulation of fluid over the cerebral hemisphere, the flow of which into the medullary region is impeded by the cerebral peduncle and the subcerebral vessels and sinuses. Thus many experiments are negative especially with the dog on his side.

*Depressor extracts supposed to have a specific effect on formation of cerebrospinal fluid*

The depressor effect of extract of nervous tissue is as marked as that of any other extract (1); in addition it is said to have a specific action on the increase of fluid (2) independently of the blood pressure changes. Dixon and Halliburton (4, p. 225) state: "This"—the brain tissue—"is the only tissue extract so far examined with the exception of choroid which gave a positive result on the cerebrospinal outflow." Frazier and Peet (2, p. 478) state: "The experiments with brain extract demonstrate that there is an actual increase in rate of flow of cerebrospinal fluid independent of the amount of fall of blood pressure."

*A priori* it is difficult to see in what respect extract of nervous tissue should so differ from other tissues as to produce a stimulation of the cells forming the fluid, for it is clear that the authors referred to above believe that brain extract stimulates fluid formation.

In order to satisfy ourselves regarding the effects of nervous tissue, fifteen injections of extracts of nervous tissue were made, including cerebrum, cerebellum, medulla, cord and pineal body. The changes produced were very similar for each of the extracts, and therefore only the effects produced by the cerebellum, which was typical for all, will be discussed in detail.

Six injections of cerebellar extracts were made into five dogs. In experiment 1 the injection produced a depressor effect on the arterial system, but the falls in venous and fluid pressures were slight. The final readings in all the pressures were lower than the initial ones. In experiments 2 and 5 injections of 5 cc. were made. In both cases there was a fall in arterial pressure with a comparable fall in venous and fluid pressures. Immediately following these injections there was an in-

TABLE 5

*Extracts of cerebrum, cerebellum, medulla, cord, pineal, cerebrospinal fluid and aqueous humor*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
Cerebrum extract												
1	199	82	106				71	43	67	113	91	122
2	82	48	90	73	46	80	82	70	90	111	94	108
3	146	132	148	134	130	132	61	61	61	56	58	58
4	148	130	146	132	124	130	60	58	59	59	62	63
5	116	90	146	98	72	118	62	55	55	72	66	54
Average	138.2	96.4	127.2	109.2	93	115.0	67.2	57.4	66.4	82.2	74.2	81
Cerebellum extract												
1	106	72	88				67	61	55	121	100	102
2	120	76	110	88	48	80	71	57	79	64	40	65
3	110	70	96	76	44	68	67	58	76	58	35	53
4	90	48	88	80	48	68	90	66	83	108	99	100
5	148	120	148	136	120	134	51	49	61	51	50	57
6	86	48	114	74	40	96	85	68	110	85	58	92
Average	110	72.3	107.3	90.8	60.0	89.2	71.9	59.8	77.3	81.1	63.6	78.1
Medulla extract												
1	88	50	86	73	52	62	93	92	90	116	108	121
2	160	108	144	140	104	130	62	48	57	53	50	61
Average	124	79	115	106.5	78	96	77.5	70	73.5	84.5	79	91
Cord extract												
1	124	88	122	96	66	92	105	84	111	267	205	296
2	160	144	160	140	128	140	66	66	63	51	56	53
Average	142	116	141	118	97	116	85.5	75	87	159	130.5	174.5
Pineal extract												
1	118	98	86	100	86	74	90	87	85	63	67	71
2	128	74	138	106	60	116	115	85	85	102	79	76
Average	123	86	112	103	73	95	102.5	86	85	82.5	73	73.5

TABLE 5—*Concluded*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
Cerebrospinal fluid												
1	124	124	120	96	96	96	75	79	75	82	82	78
2	154	154	146	114	106	106	322	330	368	187	190	205
3	156	154	156	116	115	113	319	322	321	299	302	301
Average	144.6	144	141.3	108.6	105.6	105	238.6	243.6	254.6	189.3	191.3	194.6
Aqueous humor												
1	148	152	136				124	118	93	131	130	107
2	156	154	152	111	108	105	316	317	308	300	301	296
3	124	120	122	98	94	94	91	94	95	148	154	152
Average	142.6	142	136.6	104.5	101	99.5	177	176.3	165.3	193	195	185

itial temporary rise in venous and fluid pressures of 3 mm. without any apparent rise in arterial pressure. Here the final arterial pressure was the same or less while the final venous and fluid pressures were greater than the initial pressures. This rise in fluid pressure cannot be interpreted as secretion because venous pressure rose also. In experiment 4 there was an initial temporary rise followed by fall in all the pressures. The general arterial pressure returned to normal but all the others remained slightly below normal. In experiment 6 there was a decided fall in all the pressure tubes; the final readings in each case were higher than the initial ones.

A study of the table will bear out the statement already made that the changes produced by cerebellar extracts are repeated with but slight variation by all the extracts of nervous tissue. It will further show a very marked similarity between the changes produced by extracts of nervous tissue and those produced by the depressor group previously discussed. The close correspondence of venous and cerebrospinal fluid pressures was very evident throughout. These pressures usually changed in the same direction as did the arterial pressure, but where the venous pressure did not follow the arterial neither did the fluid pressure follow it. In fact in every case the venous and fluid pressures ran almost exactly parallel. Allowing for error in the method, there was no evidence whatever of any increase in fluid that might be interpreted as secretion.

Whatever the significance of the initial temporary rise preceding the pressor effect may be, it certainly gives additional emphasis to close relationship between fluid and vascular changes. Though the rise in arterial pressure is slight and of short duration, it is sufficient to produce corresponding changes in the fluid by means of the venous change; that it is the venous pressure that here affects the fluid pressure is shown by the fact that the changes take place almost simultaneously in venous and fluid pressures and a little later than the alterations in the

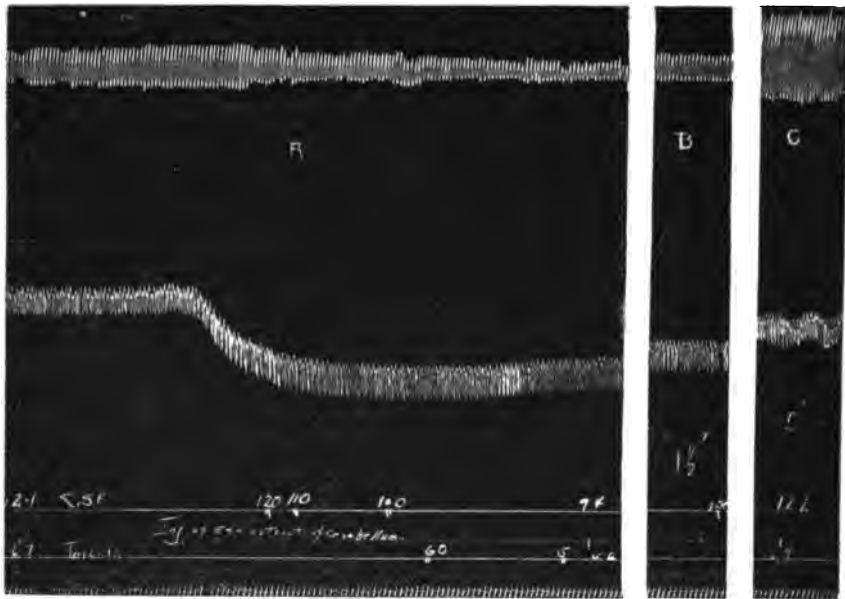


Fig. 11. This figure shows the effect of the injection of 5 cc. of cerebellar extract. *B* is taken  $1\frac{1}{2}$  minutes after the end of *A* and *C* 5 minutes after *A*.

arterial pressure. It has already been shown that a rise in fluid pressure does not produce a rise in venous.

The average of all the changes in each of the pressure tubes is as convincing here as with kidney extract, in showing that the fluid reflects vascular changes. The averages show a distinct fall in each of the pressures followed by an average return to normal with the exception that venous pressure was less than the average for the initial reading.

We are sure that they observed an actual increase in the outflow of the fluid under influence of nervous tissue extracts, but we cannot con-



cur in their conclusions. In the first place they have based their conclusion for a mechanical outflow on the observation, which our work would show to be incorrect; namely, that there is a rise in venous pressure corresponding with the fall in arterial. A study of their tracings, figures 9 and 10, shows unmistakably that the increased outflow accompanies the vascular change. Had they measured venous pressure simultaneously with arterial in their actual experiments they would undoubtedly have observed a corresponding fall in the venous pressure, a fall which would have made increased outflow possible as described earlier in the paper. In figure 11 we show a typical graph.

Dixon and Halliburton (4) likewise claim a specific action for brain extract but their evidence is less convincing than that of Frazier and Peet. They do not even show the arterial pressure on their tracing (fig. 4), hence one cannot even guess what factors may have been concerned in producing the slight outflow they record.

#### *Extracts of choroid plexus*

The extract from the choroid plexus is another depressor extract for which a specific action is claimed, in causing an actual increase in the fluid. In order to determine the effects of this extract by the manometer method, twelve injections were made into eight different dogs. The extracts were all made from fresh dog choroids, with the exception of one, which was made from fresh sheep choroids. Following seven of these injections, there was but a slight vascular change; the remaining five gave a quite marked depressor effect.

In experiment 1 the extract from one choroid was injected. There was no appreciable change in arterial pressure but there was a rise in venous and fluid pressures. In experiment 2 the injection of 10 cc. choroid extract produced practically no effect on arterial pressure, but venous and fluid pressures rose slightly. The final readings of the latter were higher than the initial readings. The second injection in experiment 8 gave a slight fall in arterial pressure accompanied by a transitory rise in venous and fluid pressures. This is the only well-marked example of a result such as Dixon and Halliburton call the usual one. (See fig. 12). The usual result is one with all pressures falling as is usual with tissue extracts. (See fig. 13). These pressures fell slightly but again the final readings were higher than the initial ones. Neither of the injections in experiment 5 produced any appreciable effects on arterial pressure—the changes in venous and fluid pressures were slight

but entirely comparable. The injection in experiment 3 (2 cc.) gave little arterial change with slight changes in venous and fluid pressures; again the latter were quite comparable. The injection (2½ cc.) in experiment 10 produced quite a marked depressor effect on the arterial side with a corresponding fall in venous and fluid pressures. All of the final readings were lower than the initial ones. The injection (2 cc.) in experiment 7 gave little arterial change with slight changes in venous

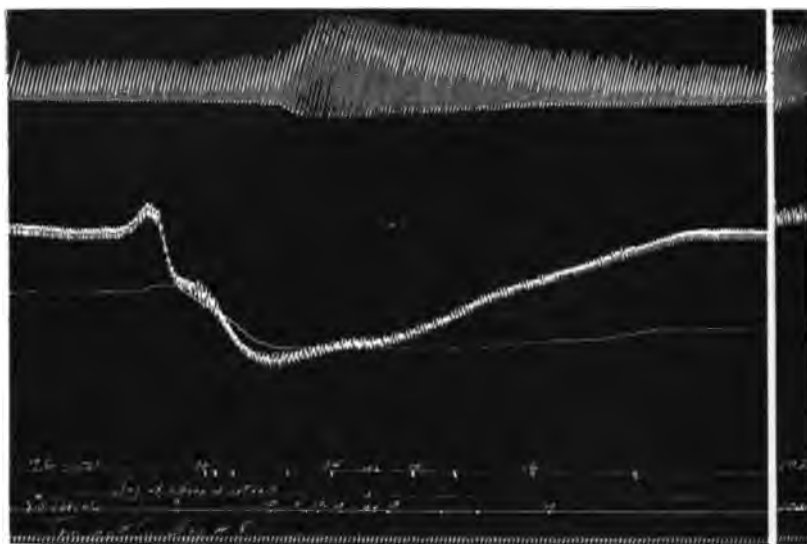


Fig. 12. This figure shows the result of an injection of choroid extract in which there was a marked fall in arterial pressure accompanied by an appreciable rise in venous and fluid pressures. Note that the maximum in each of these two pressures occurred after the maximum fall in arterial pressure. Later all the pressures rose to above normal, the change in venous pressure being adequate to explain the change in fluid pressure.

and fluid pressures, the latter being quite comparable. The first injection (2 cc.) in experiment 9 gave a decided fall in arterial pressure, accompanied by a fall in venous and fluid pressures. Here again all the final readings were less than the initial ones. A second injection (exper. 4) produced quite similar changes, except that they were less marked; in this case the final readings of the venous and fluid were higher than the initial ones. The injection in experiment 8 produced a very marked fall in arterial pressure. Almost simultaneously with the

low point in arterial pressure, the high point in venous and fluid pressures was reached; then with the rise in arterial pressure there was a fall in venous and fluid pressures, but they remained higher than they were before injection. (See fig. 12). This is the *one* experiment in our series which produced a well-developed effect, a change such as Frazier and Peet describe and which they claim is typical. The injection of 2 cc. sheep's choroid in experiment 11 gave a fair depressor effect on arterial pressure with an accompanying decline in venous and fluid pressures; the changes in the latter were entirely comparable. The injection of sheep choroid extract in experiment 12 produced a decided

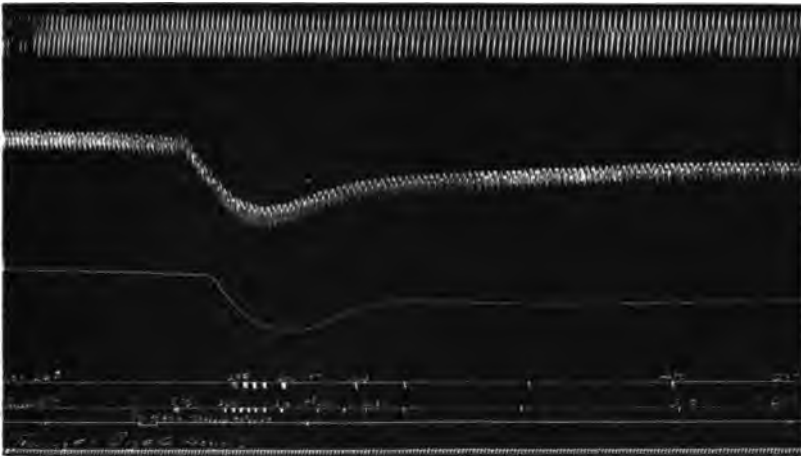


Fig. 13. This figure shows the result of the injection of 2 cc. of choroid which was followed by a fall in all the pressures under observation. Later all the pressures returned almost but not quite to the normal. Contrast this with figure 12.

fall in arterial pressure together with entirely comparable changes in venous and fluid pressures.

Reviewing the action of the injections of choroid plexus it will be noted that in those cases where there was little or no vascular change, there was likewise little change in the fluid. Where there was a change in fluid, there was almost invariably a corresponding change in venous pressure even though no change in arterial pressures was noted. The injection in experiments 9 and 12 shows a typical change following any depressor substance, i.e., a decided fall in arterial pressure accompanied by a corresponding fall in venous and fluid pressures. The changes

produced by the injection in experiment 8 were exceptional; however, the fact that such changes as these do occur occasionally (a few typical cases in 90 injections) shows unmistakably the importance of taking venous pressure simultaneously with arterial and fluid pressures, if one wishes to draw conclusions from the effect produced by vascular changes. Accompanying the low point in arterial pressure was a decided increase in respiration; this with the high venous pressure would give rise to a condition which would favor a marked outflow of the fluid with the cannula method. Again this experiment would show the error of Dixon and Halliburton's conclusion. By taking the venous pressure in

TABLE 6  
*Extract of choroid plexus*

EXPERIMENT	ARTERIAL			CIRCLE OF WILLIS			TORCULAR VENOUS			CEREBROSPINAL FLUID		
	B	D	A	B	D	A	B	D	A	B	D	A
Choroid plexus												
1	153	154	154	118	118	117	138	140	145	143	150	158
2	120	116	120	96	96	94	75	83	78	77	81	78
3	124	118	128	94	88	96	78	84	84	79	83	86
4	128	116	136	80	67	79	246	224	261	228	211	244
5	114	110	110	94	92	94	81	77	92	95	96	105
6	110	112	108	88	88	84	95	98	88	107	109	100
7	90	88	88	64	66	62	260	264	233	128	133	131
8	138	90	140				85	134	106	96	142	102
9	146	106	126	90	59	75	272	198	245	242	194	238
10	112	86	94	94	68	80	281	189	204	107	89	91
11	112	86	118	100	78	106	102	69	83	77	68	74
12	132	66	122	118	54	98	105	58	115	125	88	106
Average	123.2	104	120.3	94.1	79.4	89.5	151.5	134.8	144.5	125.3	120.3	126.0

one animal and the fluid pressure in another, they concluded from their results that venous and fluid pressures did not run parallel. Because they observed the rise in the fluid earlier in one experiment than in the venous pressure in another, they decided that the fluid had an independent secretory pressure. The error in their method of observation has already been pointed out (6), and not only this one but practically all our experiments show conclusively that venous and fluid pressures run, within the limits of experimental error, parallel. Also we have in experiments 1, 2 and 3, table 6, instances in which there was no change in arterial but where there was rise in fluid, with a parallel rise in venous pressure.

Dixon and Halliburton concluded from an increased rate of outflow of fluid from a cannula, that there is an actual increase in the amount of fluid. They discuss the possibility of physical factors which might influence the outflow, but they do not eliminate these possibilities by actual experiment. For example, they eliminate the argument that a fall in arterial pressure might cause a rise in venous pressure in the following way: "(1) increase in venous volume will be counteracted and probably counterbalanced by decreased arterial volume, so that the change of brain volume will be negligible; (2) the fall of blood pressure is not always seen; and (3) other substances which cause a depressor effect do not increase the cerebrospinal flow." Over against their argument (1) we would place our result obtained in experiment 8 showing that a rise in venous pressure does follow a fall in arterial pressure in some cases. (2) We also admit that a fall in blood pressure is not always seen, still we would refer the reader to experiments 1, 2, 3 and 5 with choroid injection in which there was a rise in venous pressure with no change in arterial. (3) In none of our whole series of experiments with depressor extracts did we observe any indisputable evidence of increased formation of fluid, hence it would appear that if Dixon and Halliburton had eliminated all the physical factors, they would not have observed the increased flow with choroid extract without some vascular change to explain the outflow. The effect of respiration observed by them is directly opposite to that reported by us in many cases in an earlier paper, and if as they say choroid extract accelerates respiration then one should expect in many cases an increased outflow rather than a decreased. The lack of response of a second injection would depend, as has been shown, on the amount of fluid in the canal rather than on the lessened effect of the chemical substance or hormone "which stimulates the secreting epithelium of the choroid gland to become active." If the mechanical factors could account for all the outflow, then it would not be necessary to assume that atropine—a substance which inhibits the activity of organs known to have secretory activity—does not effect the choroid gland, for Dixon and Halliburton state that "the increased flow is seen equally well after the injection of atropine." This action of atropine, in the light of our own experiments, would simply be an additional argument in favor of a mechanical cause for the outflow.

There is one more fact in connection with the action of the extract of the choroid which should be mentioned. If the extract of the choroid does have an action upon the choroid plexus, the point of action is not confined to that structure. Figure 14 shows the result upon the lymph



Fig. 14. This figure shows that choroid extract acts at points other than the choroid plexus. A definite lymphagogue action is shown by the increased outflow of lymph from the thoracic duct.

flow from the thoracic duct of the injection of 2 cc. of sheep's choroid into a dog. This has been confirmed by three other experiments. In two of these cases the extract was fully as active as a 2 per cent solution of peptone.

### *Thyroid extracts*

Thyroid extract is another of the depressor extracts which is said to have a specific action, but instead of stimulating the choroid plexus to excreased activity, it is said to inhibit the flow of cerebrospinal fluid. In order to determine whether the action of thyroid was really specific as claimed, a series of nineteen injections was made into thirteen different dogs. The extracts used was made from normal and enlarged dog thyroids, from the commercial preparation and from pathological human thyroids.

Five cubic centimeters of extract from a *normal* thyroid were injected in experiment 8. A pronounced fall in arterial pressure resulted, accompanied by an initial transitory rise and then a fall in venous and fluid pressures, entirely comparable with the fall in arterial pressure. The final arterial pressure was lower but the venous and fluid pressures were both higher than the initial reading. In five other injections, including extracts from normal and abnormal thyroids, in which a distinct fall in arterial pressure was observed, the changes in the fluid were, with slight variations, the same as in experiment 8. A fall in arterial pressure was accompanied by a fall in venous and fluid pressures. The final fluid pressure always depended on the venous pressure.

The injections which had a slight or negligible action on the arterial pressure gave relatively less change in the venous and fluid pressures. In a small number of these, the fluid and venous pressures rose very slightly while the arterial pressure fell, showing again the close correspondence between venous and cerebrospinal fluid pressures.

The results observed following the injection of extracts of desiccated thyroid were quite similar to those already described. The fall in arterial pressure was accompanied by a fall in both venous and fluid pressures, and at the final reading the venous and fluid pressures stood higher than before injection.

The injection of 7 cc. of extract from a pathological human thyroid in experiment 18 gave a decided fall in arterial pressure with a comparatively small drop in venous and fluid pressure. The venous and fluid pressures rose with the arterial pressure so that the final pressures were practically identical with the initial ones. The injection of 10 cc.

pathological thyroid extract in experiment 19 gave but a slight fall in arterial pressure with a comparatively small change in venous and fluid pressures.

It will be observed from the record of the changes produced by thyroid extract that the changes are in no way different than those produced by any other one of the depressor extracts. The changes in venous and fluid pressures are entirely comparable. If additional evidence

TABLE 7  
*Extract of thyroid*

EXPERIMENT	GENERAL BLOOD PRESSURE			CIRCLE OF WILLIS PRESSURE			VENOUS (TORCULA) PRESSURE			C. S. F. PRESSURE		
	B	D	A	B	D	A	B	D	A	B	D	A
Thyroid extracts												
1	116	90	122	82	57	84	90	88	86	70	58	48
2	124	70	108	80	52	64	98	80	100	97	60	60
3	130	104	124	104	90	100	63	56	57	65	63	58
4	120	108	122	98	84	98	53	50	50	53	50	48
5	152	148	160				59	62	81	48	55	71
6	162	144	158				88	96	108	79	94	108
7	130	92	124	119	84	112	120	81	122	154	134	167
8	154	90	130	116	94	116	94	70	99	87	80	95
9	126	114	114	118	114	110	101	100	98	95	100	101
10	114	68	90	90	56	66	92	69	80	292	162	190
11	102	92	104	80	79	80	76	76	77	275	280	288
12	152	124	132	102	77	83	296	255	262	287	240	260
13	118	94	116	92	78	92	100	101	105	134	130	145
14	92	82	84	70	68	64	248	236	229	94	93	106
15	124	114	114	106	96	98	78	77	86	73	65	63
16	132	132	132	110	106	106	80	80	76	72	74	76
17	120	114	118	94	92	92	97	98	98	148	147	138
18	168	100	164				56	34	56	44	27	42
19	84	72	66				14	00	-10	7	-10	-30
Average	127.3	102.7	120.1	97.4	81.8	91.0	100.1	89.9	97.9	114.4	100.1	107.9

were desired, a study of the figure 5 showing the averages for all of the pressures should be convincing. The average change for nineteen injections shows conclusively the dependence of the fluid pressure on the vascular changes for not only is there a close correspondence between fluid and venous changes, but between the fluid and arterial as well. There is absolutely no evidence for a decrease in the amount of fluid as determined by the manometer method. Frazier and Peet's observa-



tions are that "thyroid extracts give a decrease in the rate of fluid out-flow," but the evidence that they present is not conclusive. It is true that in our work individual experiments could be cited in which there is an apparent decrease in the fluid, but other experiments could be cited in which there is an apparent increase in the fluid. An average of all the changes eliminates these exceptional cases and therefore we believe that the average rather than the individual change comes nearer showing the true condition.

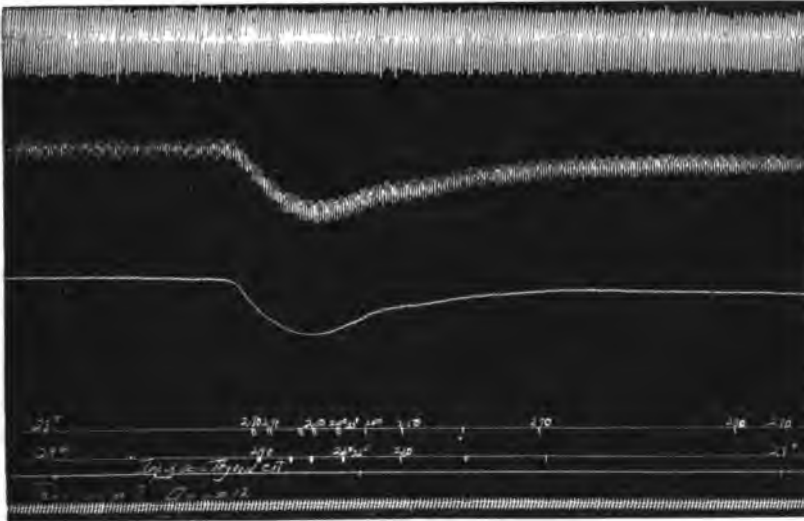


Fig. 15. This figure shows the effect on all the pressures of the injection of 5 cc. of thyroid extract. Note the fact that all pressures fell, only to return almost exactly to the normal.

Frazier and Peet (19) further state that "the decrease in rate appeared almost immediately after the injection and lasted for four and one-half hours" and that "control experiments have demonstrated that with the method we used the fluid will flow at a nearly uniform rate for this length of time." Since this method has already been fully considered, it is unnecessary to re-open the discussion here; however we must say that in the experiments with this method, our findings were similar to those of Dixon and Halliburton on this point, who report a marked slowing in the rate in the normal animals run as controls; a slowing which compares exactly with the slowing recorded by Frazier and Peet but which according to them is due to the thyroid injections.

A reference to the table shown by Frazier and Peet on the normal rate of flow (p. 275) does not bear out their statements regarding the results on their controls. They cite two animals—the first ran seven and one-sixth minutes with a marked tendency to decrease, as can be seen in their protocol at a glance; the second showed a very marked irregularity of rate and has the following note added—"stopped flowing, dog killed with chloroform." This experiment ended in eighteen minutes.

We do not doubt the observations recorded by the authors but we do not believe that in a series of ten dogs, more than one in that number would give a marked flow after three or four hours under anesthesia. This is in accord with Dixon and Halliburton who, using practically the same method, find no flow after the fifth hour.

A careful consideration of all the evidence seems to present no good reason for believing that thyroid extract causes a decrease in the amount of fluid. In the first place, control experiments show a decrease in outflow, which is in every respect comparable to the decrease in the fluid claimed by Frazier and Peet following thyroid injections. Furthermore, when the manometer method is employed, the changes following the injection of thyroid extract are no different than those produced by any one of the depressor extracts used.

It would appear, therefore, that the changes in the fluid following the thyroid injection are to be explained entirely by vascular changes and that the claim that thyroid has a specific action is not based on a true interpretation of facts.

#### CONCLUSIONS

1. Nearly all tissue extracts have a depressor effect on the vascular system; extracts of adrenal and of the posterior lobe of the hypophysis have a pressor effect.

2. Changes in arterial pressure were usually accompanied by corresponding changes in torcular (venous) pressure; there were occasionally, however, important exceptions in which changes in arterial and torcular pressures were exactly opposite.

3. The changes in fluid pressure corresponded quite closely to the venous changes. While the arterial pressure may have a direct effect on the fluid pressure, the changes in the fluid were for the most part due to the venous changes.

4. The changes in the fluid were quite comparable with all the tissue extracts having a depressor effect, likewise with those having a pressor

effect. In every case the fluid changes depended on the vascular changes, not upon new formation.

5. There is no indisputable evidence that any of the extracts used have a specific action on the fluid.

6. The changes produced in the fluid by tissue extracts are mechanical in so far as these extracts have any vascular effect.

7. Extracts of the choroid plexus have a definite lymphagogue action.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL  
SOCIETY

THIRTY-SECOND ANNUAL MEETING  
*Cincinnati, December 29, 30, 31, 1919*

*The time relations of the heart cycle as shown by the carotid pulse of man.*

WARREN P. LOMBARD and OTIS M. COPE.

The paper treated of work which was a continuation of that reported at the last two meetings of the Society. The duration of the systole of the left ventricle should give evidence of the functional condition of the muscle of the human heart. The carotid pulse tracing was used by the writers to obtain evidence of the length of the systole, because it would be of general, practical application in case the method proved of value. The time between the beginning of the rise of the primary wave and the beginning of the rise of the dicrotic wave, the P-D time, does not include the period of rising tension in the ventricle, and would, therefore, be perhaps 0.030 second shorter than the true systole of the ventricle. Nevertheless, it should give a good indication of the action of the heart muscle.

The pulse rate and the position of the subject have an important effect on the duration of the systole, and to establish a standard which should be a gauge of the condition of the heart muscle it was necessary to establish the P-D interval and the D-P interval (the time between the beginning of the rise of the dicrotic and the beginning of the rise of the primary wave), for every ordinary pulse rate when the subject was standing, sitting and lying down. This was done and, in addition, the P-D and D-P intervals were determined for subjects standing, immediately after light exercise.

A table summarizing the results, for cycle lengths from 1.200 to 0.300 seconds (pulse rates 50 to 200) was shown. Curves picturing the rate of the shortening of the P-D time, when it was plotted with cycle lengths, and plotted with pulse rates, as abscissae, and also formulae which permit the calculation of the normal, average P-D interval by men standing, sitting, lying down and standing just after exercise, were exhibited. The formulae were different from those offered at the last meeting. These results were based on 620 tests on 250 normal men, the length of the P-D and D-P intervals having been measured to thousandths of a second in more than 10,000 cycles.

It was stated that the P-D and D-P intervals are both altered by respiratory and vasomotor influences; that the P-D interval is affected more by respiratory, and the D-P interval often more by vasomotor influences; that the changes in the length of the P-D and D-P intervals

produced by the respiratory and vasomotor influences do not necessarily occur to like degrees or synchronously, and consequently are probably brought about in different ways. It was suggested that the P-D interval was influenced more through the amount of blood supplied to the left ventricle, and the D-P interval through the nerves controlling the heart rate.

The length of the P-D interval was not found to be appreciably affected by age, height or weight. The systolic, diastolic and pulse pressures, occurring within normal limits, were not found to alter the P-D interval. On the other hand, women were observed to have longer systoles, on the average, than men.

*Further observations on the relation of the central nervous system to epinephrin secretion.* G. N. STEWART and J. M. ROGOFF.

Our former experiments on the epinephrin output after transections of the spinal cord at different levels in cats have been confirmed and extended by a new series of experiments.

1. In eleven acute experiments the cervical cord was divided at levels from the fourth to the seventh segment. In several of the animals the initial output was undiminished by the section, in the others it was more or less diminished but a substantial output could still be estimated. In eight dogs (section at 4th to 6th segment) and two rhesus monkeys (6th to 7th segment) a considerable or marked diminution in output after the cord section was observed, apparently corresponding to the greater degree of shock than in cats, but even in the monkeys a substantial output could be estimated.

In three cats the brain and bulb were eliminated by ligating the arteries. It was shown by the usual tests that the anemia was complete. In all three experiments the epinephrin output was the same as before elimination of the brain and bulb, a full normal output.

2. In fifteen experiments on cats and dogs in which the animals were allowed to survive for two to thirteen days after cervical transection the output never attained the normal average and was usually much below it, although in several of the experiments the output attained a third or a half of the normal average.

3. Acute experiments with dorsal cord transections were made on a number of dogs and cats (4th to 5th segments) and one survival experiment (7 days) on a dog. The output was greatly diminished or abolished within the limits of detectability by the assay.

4. Strychnine caused a marked increase in the output both in acute and survival experiments after cervical cord transection.

5. The output of epinephrin in the above experiments was estimated by the methods previously employed by us, entailing opening of the abdomen, because we do not know of any other way at present by which quantitative estimations of the output can be made. To test the objection that opening the abdomen may profoundly alter the functioning of the adrenal secretory mechanism we have made experiments on cats with denervated heart in which the acceleration of the

heart produced by stimulation of the central end of the sciatic was compared before opening the abdomen with that obtained after it was opened and such manipulations made as would be involved in forming a cava pocket. We did not find that the reaction was essentially altered. Further experiments on cats with denervated heart confirm our previous conclusion that reactions obtained on the denervated limb and denervated heart (von Anrep, Cannon) do not constitute a demonstration of augmented epinephrin output with asphyxia and nerve stimulation until it is shown that redistribution of the blood with the ordinary output of epinephrin cannot account for the reaction.

*The influence of oxygen deficiency and related conditions upon the hemo-respiratory functions.* YANDELL HENDERSON and HOWARD W. HAGGARD.

It is generally supposed that under oxygen deficiency acids are produced in the tissues and are retained in the blood, and that the blood alkali is thus neutralized and eliminated through the urine. This may be termed the acidotic process.

Experiments on dogs subjected to progressively decreasing oxygen show, however, that the process actually involved is in many respects exactly the opposite of the usual supposition. They demonstrate that before any considerable amount of alkali is lost an abnormally large amount of  $\text{CO}_2$  is eliminated by the excessive breathing induced by a lowered oxygen pressure in the air breathed. Then alkali passes out of the blood to compensate this alkalosis. This we term the acapnial process.

From these facts and related observations on men it appears that in normal persons the blood alkali is controlled by the dissolved  $\text{CO}_2$ —more or less alkali being called into use in the blood to satisfy the normal  $\text{H}_2\text{CO}_3$ :  $\text{NaHCO}_3$  relation, and thus to keep the  $\text{CH}$  of the blood nearly constant. The amount of dissolved  $\text{CO}_2$  in the blood is controlled by the pulmonary ventilation, and fundamentally in normal persons the ventilation is adjusted to the oxygen partial pressure of the altitude at which the person lives.

When, however, over-breathing is induced and the  $\text{H}_2\text{CO}_3$  of the blood is reduced, the  $\text{NaHCO}_3$  follows downward. This, we find, and not acidosis, is what occurs also in carbon monoxide asphyxia.

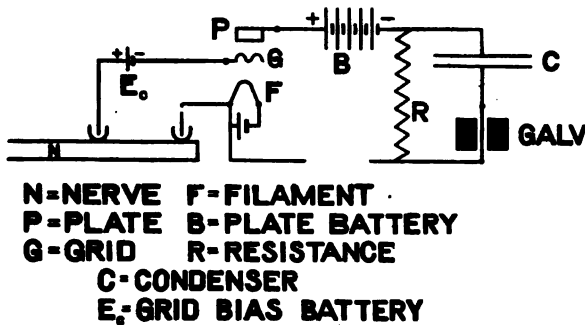
A simple test for differentiating between low blood alkali of acidotic and that of acapnial origin is the administration of air to which 8 to 10 per cent of  $\text{CO}_2$  has been added. This quickly causes death in acidotic subjects; but it induces a rapid recall of alkali to the blood, and restoration of the subject to a virtually normal condition of health if the condition is of acapnial origin.

We have now (in collaboration with Dr. R. C. Coburn) applied this treatment to patients after surgical operation and anesthesia. The result has been that alkali was recalled to the blood, that arterial pressure and other functions were restored to normal, and that the anesthetic was rapidly eliminated by the full breathing during the  $\text{CO}_2$  inhalation with a consequent great reduction of nausea.

In dogs asphyxiated with carbon monoxide (in investigations for the U. S. Bureau of Mines) strikingly beneficial results have been obtained by means of oxygen reënforced with 10 per cent CO<sub>2</sub>.

*Electron tube amplification with the string galvanometer.* ALEXANDER FORBES and CATHARINE THACHER.

Acting on a suggestion of Dr. H. B. Williams we have developed a method of using an electron tube to amplify those action currents in the nervous system, which are too small to record satisfactorily with the string galvanometer unaided. We used a "D-tube" loaned by the Western Electric Company. The device consists of a vacuum tube with a cathode in the form of a filament heated to incandescence by a current, an anode in the form of a metal plate and an auxiliary electrode in the form of a grid between the two. The hot cathode emits electrons, thus enabling a current to pass from plate to filament



within the tube if properly connected with a battery. If the grid is made positive with respect to the filament the plate-to-filament resistance is lowered; if negative, it is raised. Thus the current in the tube is regulated by variations in the potential of the grid. Small variations in grid potential can produce large variations in the plate current; thus the tube acts as a relay, reproducing greatly amplified, the impressed E. M. F.

The string galvanometer cannot be placed directly in series with the battery and the tube; the resulting current would destroy the string. At the suggestion of Mr. Sewall Cabot the string was protected by placing a large condenser in series with it, and the current was shunted by the string and condenser through a resistance and of the same order of magnitude as that of the tube. The arrangement is shown in the accompanying figure. The resistance and the tube being connected in series with a battery of fixed voltage, a change in tube resistance will cause a change in potential difference across the tube. This will cause current to flow from one plate to the other of the condenser through the string. By using a condenser of very large capacity a source of

current is obtained which is practically constant throughout the brief duration of an action current in nerve or muscle. In other words, the partial discharging of the condenser following a disturbance of equilibrium is so slow that no appreciable distortion results in the amplification of a brief action current. The battery  $E_c$  in the grid circuit is introduced to give a bias, or initial negative potential, to the grid. This serves to make the device function as an electrometer in that the excursions of the string are independent of the resistance in the tissue. It increases the amplification.

The amplification obtained with this device depends on the resistance of the tissue in circuit between the electrodes, and in part also on the resistance of the string. If the resistances of both tissue and string are very small there will be no amplification; if the resistance of either is large there will be. With a tissue resistance of 50,000 ohms about 25-fold amplification may be obtained.

A wiring scheme has been installed whereby it is possible to shift from the standard arrangement of the galvanometer to the electron tube arrangement in about two minutes, by merely throwing a few switches and making two or three minor adjustments.

The following values of the various qualities involved have been found with a "D-tube" efficient and convenient; filament current, 1.10 ampere; grid potential, -1.5 volt (one dry cell); plate battery voltage, 275; resistance, 50,000 ohms; condenser, 15 microfarads, (made up of Western Electric paper condensers).

The complete account of the work will appear soon in this Journal.

*Observations on the pathological physiology of chronic pulmonary emphysema.* R. W. SCOTT.

Two individuals with well-marked chronic pulmonary emphysema of the so-called "large lung" type have been studied over a period of several months. It was found that these patients breathe high percentages of  $\text{CO}_2$  (8 to 11 per cent) for a period of ten to fifteen minutes without any marked distress and with little increase in the minute volume over that observed while breathing room air. As an explanation of this unusual tolerance to inspired  $\text{CO}_2$ , two possibilities were considered; *a*, some interference with the passage of  $\text{CO}_2$  from the lung air into the blood; *b*, an increased capacity for the storage of  $\text{CO}_2$  in the blood and body fluids.

To study the question of pulmonary permeability, experiments were done to ascertain the rapidity with which equilibrium was established between the  $\text{CO}_2$  of the blood and the lung air. A procedure similar to that described by Henderson and Prince was employed after four or five rebreathings, the  $\text{CO}_2$  of the lung air reached a constant level which was definitely higher than that found under similar experimental conditions in normal individuals. Equilibrium was reached at the same high level and in approximately the same length of time when the air first inspired contained either a low (room air) or a high percentage of  $\text{CO}_2$  (12 to 14 per cent). A level of about 9.4 per cent of



CO<sub>2</sub> was reached in the subjects with emphysema as compared to 7.2 to 7.4 per cent CO<sub>2</sub> in the cases of several normal individuals observed. Confirmatory evidence for these results was afforded by the direct determination of the total CO<sub>2</sub> content of the arterial and venous plasma together with estimations of the H-ion concentration of the whole blood (arterial) as drawn. In the case of emphysema the arterial CO<sub>2</sub> averaged 75 vols. per cent (0°-760 mm. Hg.) and the venous CO<sub>2</sub> 80 vols. per cent. In a series of 19 normal individuals the arterial CO<sub>2</sub> averaged 57 vols. per cent and the venous CO<sub>2</sub> 63 vols. per cent. The pH of the whole blood (arterial) in the case of emphysema was normal. These facts indicate that both the free and combined CO<sub>2</sub> of the blood

are elevated but the ratio  $\frac{H_2CO_3}{NaHCO_3}$  and hence the CH is maintained normal. Apparently the degenerative process in the lung leads ultimately to diminished aeration of the blood. The free CO<sub>2</sub> is elevated, and as a compensatory measure to keep the CH within narrow physiological limits, the bicarbonate is maintained at a high level which appears to be permanent. This condition in man is analogous to that found in animals made to breathe air rich in CO<sub>2</sub>. Both cases clearly demonstrate that the bicarbonate content of the blood and body fluids is not a fixed quantity, but may undergo a considerable increase to combat an impending CO<sub>2</sub> acidosis. Since the buffer value of blood is directly proportional to the bicarbonate content, it seems probable that in the case of emphysema a higher percentage of CO<sub>2</sub> in inspired air is required to produce a given elevation in the blood CH. However, when this level is reached the ability to prevent a further increase is limited because of the definite impairment in lung ventilation in chronic emphysema.

*Observations on the physical efficiency tests used by the Royal Air Force of England.* EDWARD C. SCHNEIDER.

In England under the guidance of Lieutenant-Colonel Martin Flack a set of simple physiological tests was devised to indicate subjects who were likely to suffer from discomforts in the air, symptoms which might be due to lack of proper oxygenation of the blood and were likely to render the man unfit for flying. The tests grew in number with experience and the application was extended to detect flying stress or fatigue.

An analysis of the records of American aviators who underwent the rebreathing examination and the English set led to the conclusion that these tests do not give a reliable indication of how well a man will respond to diminished oxygen tensions. The data are not capable of physiological interpretation unless they represent the best effort that can be put into each test. Indifferent subject and listless observer both vitiate the results. The tests are in some respects psychological, since if a man determines to hold the breath until discomfort is pronounced it can be done or he may give up with the first feeling of effort. To sustain the mercury column in the "fatigue" test requires as well

some skill, and a proper set of lips may ease the performance so much that 10 to 20 seconds are added to the length of the hold. The expiratory force test requires effort and experience. One often improves his record appreciably after several performances. The mental attitude of the subject may make or mar these tests. Therefore it is believed that the tests depend too much upon the hearty coöperation and the complete attention of the subject.

*The influence of low oxygen tensions on venous blood pressure in man.*

EDWARD C. SCHNEIDER.

Low oxygen tension effects on the venous blood pressure were studied during a period of gradual decrease in which the lower limits of the partial pressure of oxygen ranged between 48 and 79 mm. Hg. The experiments lasted from 25 to 45 minutes. Three methods were employed to obtain low oxygen tensions, the low pressure chamber; rebreathing of air, from which the carbon-dioxide produced by the subject was removed by sodium hydroxide, at normal atmospheric pressure; and air diluted with nitrogen. All observations were made on officers and enlisted men at the Medical Research Laboratory of the Air Service, U. S. Army.

The effects on the venous pressure were the same regardless of the method used to vary the oxygen. This is interpreted as proof that the effects were not the result of a mechanical action but the result of the changes in oxygen.

The general response in the venous pressure during the reduction in oxygen was a fall that ranged between 22 and 146 per cent of the individual's sea-level normal. In 48 per cent of all cases the fall, which was gradual, began within the first five minutes, in which time the oxygen decreased from 21.96 to about 18.5 per cent, or from 159 to 140 mm. partial pressure. In 19.6 per cent of cases the drop first appeared between the fifth and tenth minutes or by the time the oxygen had decreased to 16 per cent (122 mm.). The rate of fall in the venous pressure was ordinarily most rapid after the oxygen reached 12 per cent (91 mm.). In 4 out of a total of 56 experiments this venous response failed to occur.

*Circulatory reactions to hemorrhage.* WALTER J. MEEK and J. A. E. EYSTER.

The reactions to hemorrhages amounting to as much as 2 per cent of the body weight have been studied in a series of anesthetized dogs by means of the X-ray, arterial and venous pressure manometers and the cardiometer. In the intact morphinized animal the diastolic size of the heart is maintained until about 2 per cent of the body weight of blood is lost. The hemoglobin determinations indicate no important restoration of blood volume during this period. Blood pressure and heart rate remain unchanged.

In the anesthetized animal with open chest and artificial respiration, the volume output per minute and the volume output per beat remain

unchanged until about 1 per cent of the body weight of blood is lost. There is then a rather abrupt decrease. Recovery after each bleeding becomes poor. Systolic and venous pressures also fail to recover their normal level and the pulse rate is permanently increased.

On the basis of these facts it may be concluded that after small hemorrhages there is some other mechanism than those usually recognized which insures a normal return of venous blood to the heart in spite of the reduced volume. A possible explanation might be found in a decreased capacity of the venous cisterns together with an increased velocity of the blood returning to the right heart. This, however, remains to be demonstrated.

*Studies on the responses of the circulation to low oxygen tension. II.*

*The electrocardiogram during extreme oxygen-want.* CHAS. W. GREENE and N. C. GILBERT.

Tests were made of the low oxygen endurance of twenty-two men by the rebreather method. Clinical records were taken at minute intervals of the pulse rate, systolic and diastolic blood pressure, respiratory rate and minute-volume. Electrocardiograms were taken at periods of five minutes during the first two-thirds of the test and at two-minute intervals in the last third. Attempt was made to secure an electrocardiogram just before the close of the experiment and a check was taken just after closing the test. Of the twenty-two soldiers tested, eight or about 36 per cent showed irregularities of the heart rate and type of beat. The heart rate accelerates rapidly toward the close of the rebreather test, a fact well established by the published experience of the Medical Research Laboratory of the Air Service. The Air Service also reports a certain percentage of cases that tend to collapse at the close of an extreme test. In our series the test was pushed beyond the usual limits, in fact to the point of inefficiency of the nerve reactions represented by impending or actual unconsciousness.

With the onset of collapse the heart rate suddenly slows. The electrocardiogram proves that the slowing is due to disturbance and suppression of function of the S-A node with temporary persistence and dominance of the rhythm by the A-V node. Conduction is also depressed if not lost as between the two nodes but not distal to the A-V node at this stage of oxygen-want. In one test complete dissociation occurred. The auricular rate was slower than the ventricular, a phenomenon not present in the usual type of heart dissociation. Complete loss of the P wave, the evidence of auricular contractions, occurred in two extreme tests. Irregularities of heart rhythm and in the point of origin of the beat were developed in at least one striking case. We conclude that extreme oxygen-want in man depresses the rhythm and the conduction in the heart and that the basal or sinus-auricular region is the most sensitive, both as to rhythm production and conduction. The mechanism is sensitive to oxygen-want in the base-apex, i.e., the auricle-ventricle direction—a point of phylogenetic significance. Doubtless the same loss holds in oxygen-want in diseases as in these normal healthy men.

*Relation of catalase to heart activity.* R. J. SEYMOUR.

Determinations were made of the catalase content of the ventricular muscle of turtle hearts which were beating at various rates as the result of exposure to various degrees of temperature. Hearts were warmed and cooled both in situ and isolated and were then tested for catalase content. The results of such determinations showed no relationship between the heart rate and catalase content.

*Removal of the duodenum.* F. C. MANN.<sup>1</sup>

The investigation was undertaken for the purpose of determining the effects of the removal of the duodenum. Previous work on the problem was reviewed. The anatomy, physiology and pathology of the duodenum and their relation to the extirpation of the organ was discussed. A one-stage operation for the removal of the duodenum was described.

The duodenum was removed from the dog, cat, hog, goat and monkey although in only the first three species has the operation been sufficiently successful to warrant conclusions. Careful studies on the dog did not reveal any noticeable changes following the duodenectomy. Some of the animals have remained in good condition for many months, and one for more than a year. In all instances in which the animal did not do well, some complication has been responsible and not the loss of the duodenum.

Examination of the blood showed it to be normal with regard to cell-counts, hemoglobin, carbon dioxide combining power and hydrogen ion concentration. None of the animals have developed glycosuria. X-ray examination has shown the course of standard barium meal to be practically the same as in the normal dog. Gastric secretion also seems to be normal. Removal of the duodenum in the hog has not prevented normal development nor normal accumulation of fat. The cat also remains in a seemingly normal condition after duodenectomy.

No data have been secured to show that the duodenum is of great importance in any of the species used.

*The interpretation of certain muscle phenomena in terms of "all or none."* THEO. KRUSE.

The interpretation of certain muscle phenomena in terms of "all or none" is based on the conception that only a limited number of fibers contract with weak stimuli. As the current is increased, more fibers become involved but the intensity of contraction of any individual fiber may be considered the same in either case.

According to this conception the height of contraction in a given state is largely dependent upon the number of fibers stimulated. Certain changes in the time relations with varying stimuli are in part due to the resistance of the non-contracting fibers. Certain changes in the time relations with varying loads are of a character similar to changes produced by weak stimuli. The principal manifestation of this change

<sup>1</sup> Preliminary report of this work has previously been made by F. C. Mann and K. Kawamura, Journ. Amer. Med. Assoc., September 20, 1919.

is a prolongation of the contraction phase so measured from the point of stimulation.

Changes in the relaxation phase due to tonus following weak stimuli may be quickly eliminated by immersion in a cocaine solution while tonus produced by strong stimuli, presumably affecting more internal fibers, may not be removed for much longer periods of immersion. The diffusion time of cocaine is believed to account for this variation.

The application of the "all or none" principle is of distinct value for the interpretation of the state of the tissue and certain changes produced by experimental procedure and by drugs. Such consideration, however, should not minimize the importance of other factors.

*Heat production in turtle's cardia in response to stimulus through vagus nerve.* CHARLES D. SNYDER.

To avoid error introduced by heat of electrical origin inevitable when smooth muscle is tetanized directly, a vago-cardia preparation of turtle's stomach is isolated and mounted in the muscle chamber for determination of heat production.

An insert thermopile of 30 iron-constantan junctions is used in series with low resistance D'Arsonval, whose sensitivity (1 mm. deflection at 1 M. distance) is  $1 \times 10^{-9}$  amp. of current. Theoretical thermometric value of 1 mm. deflection equals galvanometer sensitivity divided by amperes developed by difference of  $1^{\circ}\text{C.}$  between cold and warm junctions of thermopile, the latter in series with galvanometer of  $12 \Omega$  plus necessary additional resistance required for critical damping ( $16\Omega$ ). That is, in the present case

$$1 \times 10^{-9} \cdot \frac{12 + (12 + 16)}{30 \times 53 \times 10^{-6}} = 2.5 \times 10^{-5} ^{\circ}\text{C.}$$

The period of the galvanometer is 19 seconds, but is not too slow for smooth muscle of cold-blooded species.

As satisfactory experiments I report the two following:

*Experiment of 12/1/19.* Vago-cardia preparation; weight, 5.3 grams. Of muscle coats, only ca. 2.7 grams. Isotonic lever weight lifted by muscle in all cases 20.7 grams. Preparation left with mucosa inside, thus in direct contact with "warm junctions" of thermopile. "Cold junctions" covered with stomach muscle strips. Temperature of muscle chamber ca.  $20^{\circ}\text{C.}$

NUMBER OF TRIAL	DISTANCE OF SECOND COIL	STIMULUS	RISE OF CARDIA TEMPERATURE	CALORIES PER GRAM MUSCLE AND GRAM TENSION, ROUGHLY ESTIMATED
	cm.	seconds	deg. C.	
1	8	17	0.00165	0.00018
2	(Spontaneous contraction)		0.0017	0.00019
3	8	16	0.0014	0.00015
4	6	22	0.0005	0.00005
5	4	24	0.0016	0.00017
6	2	42	0.0017	0.00019
7	0	22	0.0016	0.00017

*Experiment of 12/6/19.* Vago-cardia preparation. Cardia everted. Weight, ca. 3.7 grams. "Cold junctions" covered with strips of stomach muscle without mucous coat. Isometric lever with Ludwig vertical writing-tip.

NUMBER OF TRIAL	DISTANCE OF SECOND COIL	TIME OF STIMULUS	MECHANICAL TENSION	RISE OF CARDIA TEMPERATURE	CALORIES PER GRAM MUSCLE; PER GRAM TENSION, ROUGHLY ESTIMATED
	cms.	seconds	grams	deg. C.	
1	8	24	13	0.0077	0.00095
2	8	33	28	0.02	0.00116
3	6	28	35	0.0083	0.00044
4	5	18	32	0.003	0.00112
5	6	21	32	0.0029	0.00101

In the two experiments here reported it will be seen that the cardia of the turtle when stimulated through its nerve exhibits a rise of temperature from 0.0001°C. per gram tension exerted in the one case, to 0.0001° or 0.0007°C. in the second case.

Whether the heat necessary for this is developed in the contracting muscle or whether a part or all of it is produced in the gastric glands of the mucosa which also are probably stimulated to activity by the fibers in the nerve trunk, cannot be stated at present with certainty and is a point that must be determined and taken into account before any further conclusions are drawn. Inasmuch as the rise of temperature at the thermocouple junctions was greater in the case where the stomach ring was everted (exper. of 12/6/19), it may be inferred tentatively that the muscle of the cardia is the greater source of heat.

*Some remarks on catalase.* THEO. C. BURNETT.

A brief review of the recent work of Burge and his collaborators is given, and the criticisms that have appeared. His theory that catalase is secreted by the liver and is responsible for oxidations is not proved. Some observations on the effect of ether on blood show that contact of fluid ether with blood is not destructive of catalase. It is suggested that catalase is a constituent of the cell itself, and varies in its composition and activity in different tissues, as shown in preparations made after the method of Batelli and Stern.

*Further studies on excitation of infections of the throat.* STUART MUDD, SAMUEL B. GRANT and ALFRED GOLDMAN.

In experiments reported last spring it was shown that chilling of the body surface causes vasoconstriction and ischemia—not, as formerly assumed, congestion—in the mucous membranes of the human palate, faucial tonsil and pharynx. In this respect these membranes and the skin evidently function similarly in heat regulation.

The chief method employed has been direct measurement of variations in temperature in the exposed mucous and cutaneous surfaces by

thermopiles applied by means of galvanized iron "applicators." The applicators in the early work were strapped in position with adhesive tape. In the present experiments they have been held stably in place by a specially devised holder; the wire applicator is held by a set screw in a groove borne by a metal spheroid which is attached by the blades of a head mirror to a second spheroid screwed into one arm of a Doyen mouth gag held between the subject's teeth. Stability and ease of adjustment are thus attained.

Although skin and mucous membranes become ischemic together on chilling the body surface, differences have been shown to exist in their reactions to rewarming. The skin returns to a little above its initial temperature; palatine and pharyngeal membranes have remained somewhat ischemic; the tonsillar temperature has risen well above its level of before chilling.

It seems possible—although this is mere speculation—that this tonsillar hyperemia is an adaptation secondarily acquired as a protection against the organisms always present upon the delicate epithelium of the tonsillar crypts.

With inhalation of amyl nitrite, skin temperature has always shown a sharp transient rise. The mucous membranes, if relatively ischemic, show a rise parallel to the skin. If already hyperemic, local vasodilation in the mucous membrane with amyl nitrite is more than counterbalanced by the lowering of general blood pressure, and temperature falls.

Daily bacteriological cultures were made from the subject's nose, pharynx and tonsils. The nasal flora was sparse and unaffected by the experiments. In three instances a chilling experiment was followed by marked relative increase in the numbers of one microorganism in the throat, the remaining flora remaining apparently unaltered. In one subject *Streptococcus hemolyticus* proliferated; in a second, *Micrococcus catarrhalis*; in a third, *B. influenzae*. The first two instances were synchronous with the appearance of a clinical sore throat; in the case of *B. influenzae* there were general malaise, slight headache and chilliness, without local symptoms.

Chilling in these experiments by exposing the bared body to an electric fan for 5.5 to 28 minutes in a room of 16.9 to 20.9°C. failed in any case to produce albuminuria or glycosuria. However, a urine more dilute than before the experiment was excreted. The reaction of the urine was usually changed from acid to alkaline; this, on further analysis by Grant and Goldman, was found to be referable to the long period of forced respiration in each experiment.

*The experimental production of edema as related to protein deficiency.*

EMMA A. KOHMAN.

Denton and Kohman,<sup>1</sup> while working on the dietary qualities of carrots, found that dropsy occurred in a large percentage of rats fed on

<sup>1</sup> Denton and Kohman: Journ. Biol. Chem., 1918, xxxvi, 249.

a carrot diet, when the proportion of nitrogen had been reduced by the addition of some nitrogenous foodstuffs, such as fat or starch. A study of the etiology of this edema was undertaken. The study was made upon rats. Diets deficient in protein, in calories, in fat-soluble-A and water-soluble-B except as they came from the carrots, and deficient in fat, respectively, were fed. Diets of high and low water content and of high and low salt content and of an increased acid content were fed.

The following observations were made:

1. When young rats are fed diets composed largely of carrots and with carrots as the only source of protein, a large percentage of the rats develop edema.

2. Fats or fat-soluble-vitamins do not prevent the occurrence or decrease the percentage of edema in rats, even if 10 per cent of the calorie value of the diet is made up of butter-fat.

3. The water-soluble-vitamins do not prevent the occurrence or decrease the percentage of edema in rats fed the low protein diet.

4. Salts do not play any appreciable rôle in the production of this type of edema, for even when the salt content is doubled there is no noticeable effect upon the occurrence of edema.

5. The water intake of the animal which is feeding on the low-protein-carrot-diet decidedly influences the development of the edema. Edema develops more frequently, is more severe and develops sooner on a wet diet than on a dry diet.

6. When a sufficient amount of an adequate protein is added to the low-protein-carrot diet, in place of an equivalent amount of corn starch, edema is not only averted in rats feeding on this diet but the rats show normal growth and reproduction.

7. That the edema does not develop as a result of some toxic substance in the carrots is shown by the fact that rats fed on the control diet with the same carrot content as the low-protein-carrot-diet but with adequate protein did not develop edema and showed normal well-being.

8. When the acid content of the diet was increased the percentage of rats developing edema was increased from 50 per cent to 80 per cent. That the acid in this diet was not the chief etiological factor of the edema is shown by the fact that one rat on this diet was cured and made to grow normally on the diet with no change in the acid content but with an adequate protein supply replacing an equivalent amount of corn starch in the diet.

9. The edema manifested in these rats is not due to simple starvation, or low calorie intake, for when rats are fed a diet, including 1 gram of adequate protein each day, of the same calorie value as the diet fed the edematous rat, they do not develop edema.

10. This edema can be successfully cured by supplying the diet with adequate protein. Edema was produced and cured three times in one rat by alternating low-protein diet (edema producing) with an adequate protein diet.



11. These findings warrant the general conclusion that if it is necessary to limit the amount of protein in a diseased condition or in a period of national economic stress (as was necessary in some of the European countries during the recent war), it is advisable to administer the low-protein diet in a form free from excess of water and any acid-producing foods. Symptoms of developing edema must be looked for and adequate protein supplied immediately to effect a cure.<sup>2</sup>

*On the origin of the muscular tremors, clonic and tonic spasms in parathyroid tetany.* A. B. LUCKHARDT, M. SHERMAN and W. B. SERBIN.

Our dogs suffered parathyroidectomy after the animals had fully recovered from transection of the cord at various levels as gauged by the condition of the animal, the wound, and the reappearance of the several spinal reflexes.

Recalling the fact that the neuromuscular phenomena without spinal transection are less severe in the hind than in the fore limbs we record our observations, in general, as follows: *a*, The fibrillary contractions occur in the musculature posterior to the transection; for they may be present in the hind limbs independently of the fore limbs; and they persist in the hind limb isolated, as far as the musculature is concerned, from the remainder of the body; *b*, The clonic spasms, likewise, persist; *c*, The tonic spasms are less pronounced. The tonic spasms, when present, are in a great measure due to the stimulation of sensory nerves of some part of the animal posterior to the transection.

There is a great increase in the reflex irritability of the animals just prior to and during the attack of tetany followed by a corresponding nervous depression after subsidence of the attack; for *a*, removal of the parathyroids simultaneously with spinal transection leads to an earlier return of the reflexes and thus cuts short the period of spinal shock; *b*, during an attack of tetany the reflexes are all exaggerated and some appear pro tempore which have not been seen before or after the attack; *c*, lack of reciprocal inhibition in the patellar phenomenon during the attack of parathyroid tetany points in the same direction.

With small graded doses of strychnine a condition can be produced in an animal indistinguishable from an attack of parathyroid tetany. Again, parathyroidectomized animals are, early during tetany, more susceptible to strychnine than before the parathyroids are removed.

Two dogs which had suffered extensive unilateral extirpations of the cerebellum some 5 weeks previously showed tonic extensions of the limbs of the homolateral as well as the contralateral side during an attack of parathyroid tetany in one instance. In the other dog the fore leg of the homolateral and the hind leg of the contralateral side were tonically extended. The tonic spasm is not as sustained as on the contralateral side. The cerebellar arc seems, therefore, to reinforce but not initiate the tonic spasm. Fibrillary contractions and clonic spasms occur equally well on both sides of the animal (fore and hind limb).

*Some observations on dark adaptation of the peripheral retina.* M. DRESBACH, JOHN E. SUTTON, JR. and S. R. BURBAGE.

The object of this report is to present some observations made on the course of dark adaptation in the peripheral retina and on the mooted question of regional differences in the retina's sensibility. A flash method was used, permitting of quantitative expression of the sensibility at any moment. The size of the field ranged from 1 to 10 degrees. The nasal and temporal regions in the horizontal plane were most frequently studied, but fields in various other meridia were also stimulated. These areas were located generally 10 or 15 degrees from the fovea. It was found that when the normal retina is well adapted to daylight and the light is suddenly and completely shut off, the sensibility, measured within a half-minute thereafter is already rapidly rising. It is doubled or trebled within the first two or three minutes, then there follows a retardation for three to six minutes, during which time fluctuations in the threshold occur. At the end of six to ten minutes, on the average, in the individuals studied, the curve abruptly rises and continues to ascend, generally with irregularities, for about an hour. We have not been able to show much increase beyond that time. The rate at which the curve rises to the maximal point varies in the same individual on different days and it varies in different individuals. Often there is a marked decrease in the sensibility at the end of an hour, or sooner. Marked fluctuations may occur at any time during the observations and these fluctuations cannot always be ascribed to fatigue, autokinetic sensations associated with fixation of the eye in total darkness or other disturbances commonly met with in work of this character. They seem to be due to actual changes in sensibility of the cerebro-retinal apparatus.

Regional differences in sensibility exist. The difference between the nasal and temporal sensibility in equal fields 15 degrees from the fovea, e.g., may amount to as much as 60 per cent at any instant. At another time the same regions in a given individual may have the same threshold value.

*Paradoxical pupil dilatation following afferent path lesions.* JOSEPH BYRNE.

All previous contributions refer to efferent path lesions. In the present studies paradoxical effects were elicited by adrenalin intravenously, death, asphyxiation, etc., as follows: (1) In the contralateral pupil after *a*, section or alcoholic injection of one sciatic nerve; *b*, section of dorso-spinal roots Lvii-Sii; (2) In the homolateral pupil after *a*, hemitranssection of the spinal cord in upper or lower cervical, or in lower thoracic or upper lumbar segments; *b*, section or alcoholic injection of the brachial plexus; *c*, section of the posterior spinal cervical roots. Paradoxical effects are not abolished by cord transection just above or below the upper thoracic segments, nor by section of the cervical sympathetic, and are not prevented from appearing by cervical cord transection. After sciatic section, etc., paradoxical effects may be elicited from about the 8th to the 15th day, being maximally in evi-

dence about the 12th day. Conclusions: (1) As the histological changes, degenerative and regenerative, that take place in the dorsal root ganglia after sciatic section closely coincide in time and extent with the appearance and disappearance respectively of the paradoxical pupil phenomena, it seems these latter are conditioned by the functional activity of the neurone bodies of the dorsal root ganglia and more especially of the smaller neurone bodies which give origin to the unmyelinated, pain-bearing fibers of the peripheral nerves. (2) From all regions of the periphery in the waking state a flow of afferent impulses impinges on the upper thoracic segments conditioning the efferent flow from the cord to the dilator pupillae. (3) The efferent flow from any region of the periphery probably affects both pupils but because of unequal anatomical distribution one pupil is more affected than its fellow. (4) No evidence has been found of the existence of cerebrospinal dilator pathways in the cervical spinal cord.

*A comparison of the physiological effects of alpha and beta rays.* ALFRED C. REDFIELD.

A study of the effects of alpha rays from radium upon the egg of *Nereis limbata* indicates that there are no fundamental changes produced by alpha rays which are not also produced by beta rays. Differences in the effects of alpha and beta radiation can satisfactorily be explained by differences in their penetrating power. Whereas the beta ray passes through the egg with slight loss of energy, and acts with almost equal intensity on all parts of the cell, the alpha ray is completely absorbed before passing two-thirds of the way through the egg, and acts with far the greatest intensity upon the surface layers of protoplasm on the side of incidence. Consequently changes in the process of jelly secretion and membrane formation are limited to the side of the egg exposed to alpha rays.

Unfertilized eggs may be activated with alpha rays, but not with beta rays. This difference is explained by the fact that in the case of alpha radiation the cytolytic action of the rays is limited largely to the cortex, while the interior of the cell is little damaged. With beta rays the interior of the cell is killed before cortical cytolysis has proceeded far enough to induce activation.

Eggs exposed to a mixed beam of alpha and beta rays are killed only slightly more readily than if the alpha rays are excluded from action by means of a suitable filter. This is because the alpha ray effects are sharply localized at the point of absorption and the protoplasm which is not reached by alpha rays survives unless the accompanying beta radiation is strong enough to kill it.

*Microdissection studies on the fertilization of the starfish egg.* ROBERT CHAMBERS.

When removed directly from a ripe ovary the egg of the starfish possesses a large spherical nucleus or germinal vesicle. After the egg has stood for a while in sea water the boundary of the nucleus fades from view and the nuclear sap begins to diffuse throughout the egg

substance. A small remnant of nuclear material persists as the definitive egg nucleus which produces the polar bodies and, upon fertilization, unites with the sperm nucleus to form the segmentation nucleus.

It has been shown that non-nucleated fragments of the egg in the germinal vesicle stage are non-fertilizable. On the other hand, any fragment of an egg in which the germinal vesicle has disappeared will admit sperm and undergo segmentation.

The experiments recorded here constitute an attempt to trace the distribution of a substance which is necessary to render the egg fertilizable and which appears to emanate from the egg nucleus.

Non-nucleated fragments were cut from eggs in the various stages of the disappearance of the germinal vesicle and sperm was added. The ability of the fragment to approximate regular segmentation was in direct proportion to the amount of intermingling of nuclear sap and egg substance before the fragment was cut off. The later the stage in the disappearance of the germinal vesicle the less irregular is the segmentation of a fragment.

This substance which renders the egg capable of fertilization finally accumulates in the periphery of the egg. It was possible to tear the surface layer of the egg and to cause almost all of the internal protoplasm to flow out through the tear and form itself into a spherule which pinches off from the rest of the egg. The remnant, consisting largely of the more solid ectoplasm, tends only slowly to round up. The extruded very fluid mass immediately assumes an almost spherical shape. This may be termed the endoplasmic sphere. The fragment consisting of the ectoplasmic remnant is readily fertilizable and undergoes normal segmentation. The endoplasmic sphere is non-fertilizable no matter whether it contains the egg nucleus or not.

If the endoplasmic sphere remains connected for some time with the ectoplasmic remnant, it is fertilizable. The ability of the endoplasmic sphere to approximate normal segmentation is a function of the length of time that it remains in continuity with the original ectoplasmic mass. This is intelligible by assuming that the substance which renders the egg fertilizable tends to distribute itself over new protoplasmic surfaces.

The conclusion is that a substance emanates from the germinal vesicle or nucleus of the immature egg, diffuses gradually throughout the substance of the egg until it accumulates in its periphery and on long standing in sea water may be lost from the egg. The presence of a sufficient amount of this substance in the protoplasm of the egg is necessary to fertilization.

*Susceptible and resistant phases of the dividing sea-urchin egg when subjected to various concentrations of lipoid-soluble substances, especially the higher alcohols.* FRANCIS MARSH BALDWIN.

When subjected to suitable concentrations of various lipoid-soluble substances—i-amyl, hexyl, heptyl, octyl and capryl alcohols—the developing sea-urchin egg shows unmistakable rhythms of susceptible and resistant phases, which facts when taken in connection with the

earlier observations of Lyon, Herlant, Mathews, Spaulding, Lillie and others, constitute additional evidence that a very intimate relation exists between the general physiological condition of the egg and the physical state of its plasma-membrane. During the first few minutes (ten to fifteen minutes after fertilization) the eggs are more susceptible to all substances tried than at any other time until the period just preceding and during the division process. A comparatively resistant phase gradually becomes more and more evident up to just before the cell-division (between forty-five and forty-eight minutes after fertilization). A period of marked increased susceptibility occurs during the division process which outlasts the furrow formation in most cases by several minutes (some instances by as much as ten or fifteen minutes), and during this interval marked cytological effects in the eggs are noted. Subsequently the resistant phase is largely recovered, and is then maintained to a greater or less degree up to the time of the second cleavage.

The most favorable concentrations of the various alcohols for demonstrating the rhythmical relations range as follows: i-amyl, between 0.7 and 0.9 vol. per cent; hexyl, between 0.13 and 0.17 vol. per cent; heptyl, between 0.06 and 0.07 vol. per cent; normal octyl, about 0.015; while capryl was considerably above its isomere (normal octyl), in toxicity varying in range between 0.035 and 0.045 vol. per cent. The best records were obtained using i-amyl and capryl alcohols, possibly indicating a higher specific toxicity of these when compared to the others. When suitable concentrations were used, no marked differences could be detected by varying slightly the durations of exposure. Eggs exposed for five, eight or even ten minutes to the same concentration gave similar results when referred to survivors. This however would apply only within comparatively narrow limits. Data when plotted with reference to survivors and time exposures respectively give typical reversible curves in practically all cases.

*Effect of breathing dry and moist air.* E. P. LYON and ESTHER GREIS-HEIMER.

The air surrounding the body of human subjects was kept constant in temperature and humidity, while these subjects for 10 to 15 minute periods breathed air of constant temperature but varying humidity. Temperature of room, 20 to 22°C., humidity of room, 50 to 60 per cent. Temperature of air breathed 20° to 30°C. in different experiments; humidity of air breathed, about 10 per cent and 90 per cent respectively. Tentative results: higher pulse rate, lower respiration rate, higher arterial pressure, greater peripheral vascularity in breathing moist air than in breathing dry air.

*Acidosis a criterion of surgical shock.* BERNARD RAYMUND.

The work here summarized is an attempt to determine whether there might be a so-called shock level in the alkali reserve, such as would constitute, in experimental work, a useful criterion of surgical shock.

Experimental procedures were those commonly employed. Dogs under ether anesthesia were brought into a state of shock by gentle manipulation of the intestines for short periods of time only. The criteria for shock used were those proposed by Mann in 1914 and since generally adopted. The following conclusions were arrived at:

1. The normal alkali reserve of the dog, as determined on the venous plasma by the Van Slyke method, varied between 32.4 and 59.5 volumes per cent, corrected. The mean of sixty-two determinations was 42.7 volumes per cent.

2. The effect of etherization was to cause a mean fall in the alkali reserve of 10.5 volumes per cent from the normal. If etherization is prolonged up to three hours the mean fall from the normal is 21.2 volumes per cent. Control experiments showed that there is not much further change in the alkali reserve if etherization is prolonged still further. Thus after ten hours of continuous ether anesthesia one dog showed a fall in the alkali reserve of 13.8 volumes per cent, while another showed a fall of 25.6 volumes per cent. These figures all refer to the arterial plasma.

3. The dogs used could be classified on the basis of the length of time they survived after trauma was begun. Thus four types of shock were made out, ranging from the more severe, characterized by sudden onset and death, to that in which few or none of the cardinal signs were observed. The majority of the dogs showed the intermediate types, living from two hours (type II) to four hours and a half (type III) after beginning trauma.

4. When shock ensued suddenly the fall in the alkali reserve was relatively insignificant. When shock was slow in appearing the alkali reserve might remain high for some time, not falling until after the condition of the dog had become quite grave. There was no correlation between the degree of this fall and the type of shock; nor could the resistance to trauma be predicted from the dog's initial alkali reserve.

5. Our experiments showed further that the alkali reserve may fall no lower in shock than in previously conducted ether controls from which the dogs make good recovery.

6. Thus there is no critical level for the alkali reserve of the blood in relation to traumatic shock.

7. None of the signs of shock were shown by dogs or cats given lethal injections of acid or acid phosphate.

*Further results on the physics of sphygmography.* A. M. BLEILE and CLYDE BROOKS.

The Riva-Rocci and similar types of sphygmomanometers contain certain elements of construction which do not permit the determination of systolic and diastolic pressure by direct reading of the manometer oscillations.

One factor lies in the large volume of air in the cuff relative to the small volume of displacement possible by the restricted brachial artery. The compressibility of the air and consequent change in volume would

set a limit to pressure given by the manometer which is dependent on the possible limited change in the volume of the artery rather than on the pressure itself. Boyle's law  $PV = K$  where  $P$  = pressure,  $V$  = air in the cuff and  $K$  the resulting constant, applies here.  $P$  being large (750 mm. atmos. pressure and say 100 mm. added = 850).  $V$  being large, usually about 200 cc. or 200,000 cu. mm. the product would be 170,000,000. Now  $P = \frac{K}{V}$  and the pressure obtainable by pulsation

of the brachial would at the most be the addition of the volume of that artery which, assuming a diameter of 5 mm., length of cuff of 100 mm., would be about 2,000 cu. mm. which sum only would be subtracted from the divisor thus giving a relatively small possible difference.

Even without this theoretical treatment the general fact was recognized and this led to other methods than direct reading of the manometer such as interpretations of the extent of the oscillations, the determinations of pressures at the extinction of the radial pulse and its reestablishment by palpation and, later, by determining these points by the auscultation with a stethoscope placed over the brachial artery just below the cuff. Without discussion of these methods we offer another which seems to have certain advantages. The cuff is filled with water, a non-compressible fluid as compared with air; one tube of the cuff is connected with a three-way tube again uniting into a single arm connected with the manometer. One branch of the tube when open gives the ordinary manometric readings, if its valve is closed to a capillary opening the mean arterial pressure is given. Another branch carries a valve opening to the manometer and with this tube open the maximum or systolic pressure is given. The third branch carries a valve opening from the manometer toward the cuff and when this is open the minimum or diastolic pressure is obtained. All pressures are of course influenced somewhat by the resistance of the overlying tissues. The cuff is filled with a pressure bottle which can readily be lowered thus taking off the pressure when discomfort is felt by the subject and raised after recovery without derangement of the apparatus, thus making long observations possible.

*The influence of internal secretions on blood pressure and the formation of bile.* ARDREY W. DOWNS.

A study was made of the effect of intravenous injections of the following gland substances on blood pressure and the formation of bile: Mammary, orchic, ovarian, pancreatic, splenic, thymic and thyroid. To this list was added secretin and solution of adrenalin chloride.

As the result of these experiments we believe that some at least of the endocrine organs exert a specific influence on the secretory activity of the hepatic cells leading to the production of bile. The output of bile in the dog is increased by the administration of secretin. It is decreased by the administration of adrenalin, and by mammary, orchic, ovarian, pancreatic and thymic gland substances. It is not affected in a constant or definite manner by the substance of the spleen

and thyroid gland. Blood pressure is raised by adrenalin and lowered by pancreatic substance and the secretin preparation employed. A fall of blood pressure, ordinarily preceded by a slight rise, is caused by orchic and mammary gland substances. Oscillations of blood pressure are caused by ovarian and thyroid gland substances. Blood pressure is not usually affected by splenic and thymic gland substances.

While the experiments are too few in number to permit definite conclusions to be drawn yet it seems certain that there is no constant relation between blood pressure and the amount of bile secreted. Adrenalin consistently raised blood pressure and lowered bile formation. Secretin, where it caused a change in blood pressure, produced a lower pressure and a great increase in the flow of bile. It might be urged that thyroid gland substance owes any action it exerts upon blood pressure and bile formation to the intervention of the adrenals; this cannot be entirely controverted by our experiments. That thyroid substance increases the output of adrenalin has been shown by Bückner,<sup>1</sup> Rudinger, Falta and Eppinger<sup>2</sup> and Gley and Quinquaud.<sup>3</sup> In our experiments there is no constant relationship between blood pressure and bile production after the administration of thyroid gland substance. We did not find bile production regularly decreased when blood pressure rose or vice versa. Intravenous administration of the substance of the pancreas caused lowering of the blood pressure and lessening of the output of bile. To a lesser extent thymic substance acted in the same manner. If we place in contrast with this the effect of secretin it would seem that we are not justified in concluding that the effect on bile formation is due to the alteration in blood pressure.

*The blood in clinical shock.* G. C. WEIL and C. C. GUTHRIE.

These studies are an extension of the blood studies in experimental shock, previously reported.

The material has consisted of seven patients admitted to the hospital following traumatic injury, as gun shot wounds or crushing of the limbs, and one post-operative case. The clinical symptoms of those in shock varied from mild to the most pronounced, death occurring in some instances.

Blood samples were taken by ear puncture and from an arm vein by means of a syringe.

*Red cell counts* were of subnormal to normal range. Ear samples (by puncture, i.e., capillary) ranged slightly lower than venous.

*Hemoglobin* (by Sahli's method) was concordant with red cell counts. This was the case also for the hemoglobin content of venous blood as calculated from the oxygen capacity (Van Slyke's method).

*Plasma bicarbonate* (by Van Slyke's method) was in the upper limits of the subnormal range.

<sup>1</sup> Bückner: Compt. rend. d. l. Soc. d. Biol., 1908, lxiv, 1123.

<sup>2</sup> Rudinger, Falta and Eppinger: Zeitschr. f. klin. Med., 1908, lxiv, 1.

<sup>3</sup> Gley and Quinquaud: Compt. rend. Acad. d. Sci., 1913, clvi, 2013.



*Conclusions:* The results accord with previous experimental findings,<sup>1</sup> namely, as indicated by red cell count and hemoglobin concentration, the blood was of subnormal to normal dilution.

The plasma bicarbonate content was moderately diminished.

Causatively, blood changes are not considered significant.

Drs. W. S. McEllroy and T. K. Kruse are associated with us in this work.

*A method for determining the rate of oxygen absorption by blood.* W. S. McELLROY and C. C. GUTHRIE.

Blood from influenzal cases was observed to be of dark color and, as judged by the hue, resistant to oxidation on exposure to air. Addition of serum from the same blood accelerated arterialization but not so greatly as normal serum. In serum of some bloods of this type methemoglobin was detected spectroscopically. It was considered desirable to determine if the phenomenon was related to possible deficiency in oxygen-transmitting capacity of the serum.

The method presented has been developed from this standpoint, with particular reference to the rate of oxygen absorption. Briefly, it consists in reducing blood (1 cc.) to approximately normal venous oxygen content by means of a partial vacuum, and then continuously shaking it in a closed air chamber until it is saturated. Changes in volume of the air in the chamber are followed by frequent readings of a delicate manometer. Quantitatively, the results closely agree with direct oxygen content determinations (by Van Slyke's method), and the curves, plotted from the readings, correspond well with curves of the rate of absorption established by other methods. Control tests show comparatively small differences.

The method is in the experimental stage and, therefore, all factors concerned have not been evaluated, nor its value and limitations established, but the results encourage further study.

*Further studies on the action of acacia and associated colloids.* THEO. KRUSE.

1. The agglutination of human blood by gelatin and acacia is equally intense and is only slight with soluble starch.

2. Evidence was presented showing that agglutination by certain colloids seems to be a function of the aggregate and seems less apt to be due to a possible impurity unless its presence is proportional to the concentration of the aggregate.

3. Reasons were presented which seemed to explain the failure of agglutination of ox corpuscles by the more fixed condition of their surface charges.

<sup>1</sup> Guthrie and Guthrie: Proc. Soc. for Exper. Biol. and Med., 1914, xi, 148.

Guthrie: Proc. Amer. Physiol. Soc. for 1917; Journ. Amer. Med. Assoc., 1917, lxi, 1394; Arch. Int. Med., 1918, xxii, 1.

McEllroy: Journ. Amer. Med. Assoc., 1918, lxx, 846.

Mummery and Symes: Journ. Physiol., 1907, xxxvi, xv; British Med. Journ., Sept. 19, 1908.

4. Agglutination is interpreted as an adsorption phenomenon.
5. Experiments on rhythmical tissues with acacia give evidence of adsorption.
6. The current view of explaining colloidal responses in terms of colloidal osmotic pressure was shown to be inadequate in so far as it fails to explain certain facts relating to acacia, gelatin, dextrin and soluble starch.
7. An interpretation based upon adsorption on capillary walls was suggested for consideration since current facts dealing with these colloids are thereby explained.
8. Experiments thus far indicate that such solutions which are recommended to prolong the maintenance of blood volume, apparently do so by an interference with the normal paths of fluid exchange by an adsorption of colloid on capillary walls, and when the concentration of such colloids is of sufficient magnitude to produce this effect agglutination may play a prominent rôle.

*Observations on the capillary blood pressure in man with presentation of a new method.* D. R. HOOKER and C. S. DANZER.

A method is presented for the study of capillary blood pressure in man. It differs from all of the previous work in the criterion taken for the determinations. This depends on the visualization of the corpuscular flow within the capillary loops of the skin. For this purpose the skin immediately adjacent to the cuticle of the nail is covered with a drop of castor oil and a concentrated light is thrown on this area and the capillaries are observed through the microscope. We have found a magnification of about  $70\times$  satisfactory; this must be had with an objective giving a focal distance of not less than 16 mm.

The apparatus consists of a closed chamber having a glass roof and a floor consisting of loosely tied on peritoneal membrane or goldbeaters' skin, which is soaked in castor oil. The oil makes the membrane soft and transparent. Air is pumped into the chamber until the membrane adheres to the skin. Now the pressure within the chamber is raised until the corpuscles stop. When the pressure is slightly lowered the corpuscles begin moving again. At this point the pressure is read off on a mercury manometer. All of the corpuscular phenomena seen by Roy and Brown on the frog have been observed in man.

The capillary blood pressure was studied in 31 individuals (25 adults and 6 children). It varied between 18 and 26.5 mm. Hg.

The average pressure in a series of capillaries was remarkably constant from day to day, although the pressures in individual capillaries were variable.

Studies on postural variations of capillary pressure exclusive of hydrostatic effect showed the pressure was lowest in the horizontal, highest in the vertical and between the two in the sitting posture.

The effect of temperature was as follows: Local cold produces a fall in capillary pressure followed by a marked rise much above the normal and then a slow return to normal after the cold has been removed.

Local heat causes a rise; on removing the heat the pressure first falls to a subnormal level and subsequently returns to the normal value.

Increased intrathoracic pressure causes a rise in capillary pressure and lessened intrathoracic pressure produces a fall in capillary blood pressure.

The method is simple and can be applied clinically. We propose the name "Micro-Capillary Tonometer" for our apparatus.

*Some effects of ether and morphine on the blood and circulation in shock.*

McKEEN CATTELL.

The blood pressure of animals in shock becomes very sensitive to the effects of ether and chloroform, the inhalation of an amount insufficient to abolish the eye reflex causing a large drop in pressure. This degree of anesthesia was without permanent effect on the blood pressure of unshocked animals. The same degree of anesthesia produced by nitrous oxide and oxygen in the most favorable proportions (usually three parts nitrous oxide to one of oxygen) given to animals in this condition caused no fall in blood pressure. After a large dose of intravenously injected adrenin the ether fall was much less or entirely absent. Pituitrin was without effect. In this condition of shock after prolonged low blood pressure the vasomotor center is quite insensitive to stimulation by asphyxia, and it is suggested that this increase in ether effect may be due in part to an impairment of the vasomotor center. In the unshocked animal the decreased heart output resulting from the administration of ether may be compensated for by a reflex peripheral constriction. This does not occur in the shocked animal and the pressure continues to fall to zero.

Morphine given in large doses did not accelerate the development of shock produced by reducing the blood pressure to 60 mm. Hg. by means of pressure in the pericardium. Determination of the alkaline reserve at a shock blood pressure did not show the usual fall when morphine was given, or if it was given after acidosis had already developed, the reading returned nearly to normal. The results are summarized in the table, the figures representing the average per cent of carbon dioxide in samples of the blood plasma taken at the times indicated.

	NUMBER OF CASES	DURATION OF LOW BLOOD PRESSURE (60 MM.)			
		Start	1 hour	2 hours	3 hours
No morphine.....	8	34.7	27.1	26.1	26.1
Morphine after first hour.....	8	30.1	21.9	23.9	27.6
Morphine at start.....	6	33.5	32.2	34.0	34.7

*The rôle of the vagi and splanchnic nerves in the genesis of shock from abdominal operations.* A. C. IVY.

In dogs, both vagi were cut intrathoracically above the diaphragm and both splanchnics were sectioned and celiac plexus with the ganglion extirpated, thus isolating the intestines from the central nervous system. Such animals, if properly cared for recover quickly and live

in good health. After recovery (four to six weeks) the animals were tested under local anesthesia (novocain-adrenalin). The left carotid was cannulated for blood pressure, the abdomen opened widely and the intestines were vigorously manipulated. No signs of pain were observed except when the mesentery was pulled. Care was taken to avoid this and all other causes of pain. Samples of blood were drawn at intervals from the inferior vena cava by syringe, and hematocrite, hemoglobin, red blood corpuscle count and plasma bicarbonate determinations were made. The body temperature of the animals was followed. The same procedure was carried out on normal controls.

The following observations were made: It was possible to produce shock by vigorously manipulating the intestines of animals in which both vagi and splanchnics had been sectioned and time allowed for recovery from the operation. However, it took from three to five times longer to produce shock in the dogs with isolated intestines than the intact control animals. Also, the fall in blood pressure was much more gradual in the former than in the latter. The blood changes in both series of animals were practically the same as those reported by other observers working on shock. Marked congestion of blood and loss of irritability of the intestine to mechanical stimulation appeared sooner in the control animals than in the animals with isolated intestines.

The conclusion is warranted that the vagi and splanchnics are not directly concerned in the genesis of shock produced by manipulations of the intestines, but the presence of these nerves does hasten the onset of shock.

*The chemistry of gar roe.* CHAS. W. GREENE and ERWIN E. NELSON.

The averages of the results of the analyses of ten samples of the roe of the gar, *Lepidosteus platostomus*, were given. The water content was found to be about 55 per cent. The water content was found to be highest in the youngest, most immature samples, and was here associated with a high value for the extractive solids, and low values for proteins and lipoids. Conversely, in the older, more mature samples, the water was low, the extractive solids were small in amount, and the lipid and protein values were high.

The ether-soluble, or lipid fraction, was found to be high in value, giving an average determination of about 16 per cent, and in one case reaching the value of 28 per cent, which is one of the highest given in the literature. The proteins, determined directly, varied from 15 per cent to 28 per cent, with an average of about 24 per cent.

The extractive fraction was small relative to other tissues or compared with other fish eggs. The total solids averaged about 1.5 per cent, and the ash about 0.36 per cent. The total nitrogen of the extractives varied from 0.05 per cent up to 0.3 per cent, with an average of about 0.16 per cent. Of the total nitrogen, about 20 per cent is found in the alpha-amino nitrogen fraction, the average figure being 0.034 per cent creatin and creatinin determinations gave an average of 0.0073 per cent, varying from 0.0038 to 0.0096 per cent, determined as creatinin nitrogen.

*The excretion of a red pigment in the sweat of man.* M. H. GIVENS, V. L. ANDREWS and H. B. McCLUGAGE.

An apparently healthy, well-developed boy, age 10 years, weight 58 lbs., excreted on two occasions perspiration which contained a red pigment. Two years intervened between attacks and the duration of each was about ten days. The first attack occurred in the month of May and the second in October, the weather being rather warm each time and favorable for one to perspire.

The pigment was excreted in the sweat appearing around the left eye and down onto the cheek bone. If perspiration was evoked by either exercise or the application of heat the pigment was produced. Removing the pigment with water and subjecting the face to dry heat from an electric stove for six consecutive times markedly diminished the output of pigment. A rest of twenty-four hours sufficed to permit a new accumulation of material. The pigment colored the face a bright red resembling a smear of blood.

The dye as excreted was soluble in water and dilute alkalis and insoluble in dilute acids and ether. Addition of dilute hydrochloric acid to the face washings precipitated the dye which could then be dissolved in ether. By this procedure a small amount of material was purified. On evaporating the ether a dark red volatile oily substance was obtained. This purified material contained no nitrogen and left no residue on igniting. No phenol grouping could be detected. With 30 mgm. of material a neutral equivalent of 466 was obtained.

The possibility that diet may have played some rôle in this peculiar metabolic disturbance cannot be overlooked. At the first attack the boy had a marked craving for and consumed a large amount of lemons and at the second one there was a desire for and large amounts of tomatoes eaten. On the assumption that a large intake of organic acids might stimulate a production of the pigment, a short intensive feeding of lemons was tried after the color had ceased to appear. Negative results were obtained within the short experimental period.

*Vascular reaction to epinephrin in perfusates of various H-ion concentration.* CHARLES D. SNYDER and W. A. CAMPBELL, JR.

Reversal effect of epinephrin (Hoskins, 1912; Cannon and Lyman, 1913) was observed by Snyder and Andrus (Journ. Pharm. Exper. Therap.) when the drug was applied to isolated turtle heart. While augmenting tonus already evoked by a Ringer of 7.8 pH index, it still further diminished the atony evoked by Ringer of pH 7.0. This pointed to the possibility of the H-ion concentration as being the reversing agent. To test the validity of this view experiments were planned and some have been carried out as a special investigation.

So far fresh fall frogs prepared for vascular perfusion (Trendelenburg's method) give confirmatory evidence. The dilution of the adrenalin chlorid must be very great. If it is not more than 1:10<sup>7</sup>, only constrictor effects are obtained. We submit the following:

REMARKS	PERFUSION BEGUN AT	RECORD BEGUN AT	pH INDEX	ADRENALIN CONCENTRATION	NUMBER OF DROPS IN ONE MINUTE
Experiment of 11/25. Frog I, pressure, 19 cm. of water.		11:25	7.0	0	22
	11:29	11:34	7.0	1:10 <sup>9</sup>	28
	11:38	11:44	7.0	0	26
	11:47	11:53	7.0	0	26
	11:57	12:03	7.0	1:10 <sup>9</sup>	30
	12:10	12:16	7.0	0	24
	12:19	12:25	7.0	0	22
Experiment of 12/13. Pressure at 18 cm. water.	1:34	1:40	7.2	0	59
	1:43	1:49	7.2	1:10 <sup>9</sup>	72
	1:52	1:58	7.2	0	43
	2:00	2:06	7.2	1:10 <sup>9</sup>	65
	2:09	2:15	7.2	0	60
Experiment of 11/8/19. Eviscerated male, pressure, 10 cm. water column.		11:50 a.m.	7.0	0	2
	11:54	11:59	7.0	1:10 <sup>9</sup>	49
	12:01	12:06	7.0	0	5
	12:08	12:13	7.8	0	2
	12:16	12:21	7.8	1:10 <sup>9</sup>	1 drop in 3 minutes
	12:21	12:28	7.8	1:10 <sup>9</sup>	
	12:31	12:36	7.8	0	
	12:39	12:44	7.0	0	2
	12:48	12:55	7.0	1:10 <sup>9</sup>	5
	12:57	1:03 p.m.	7.0	0	18
	1:08	1:14	7.0	1:10 <sup>9</sup>	14
	1:19	1:29	7.0	0	22
	1:33	1:38	7.8	0	19
	1:42	1:47	7.8	1:10 <sup>9</sup>	3
	1:50	1:55	7.0	0	2
	1:59	2:04	7.0	1:10 <sup>9</sup>	32
					47

In the experiments of 11/8/19 the reversal effect is most marked—the extreme diminution of outflow during constriction being doubtless due to the low head of pressure used. It will be noted that the same dilution of the adrenalin in Ringer of pH = 7.8 not only gives no reversal (dilator) effect, but even appears to augment the constriction already evoked by the excess of OH-ions.

*The effect of the subcutaneous injection of adrenalin chloride on the heat production, blood pressure and pulse rate in man.* WALTER M. BOOTHBY and IRENE SANDIFORD.

The results of forty-six experiments on the effect of the subcutaneous injection of adrenalin chloride (0.5 cc. of 1-1000) on the metabolic rate, respiratory quotient, pulse rate and blood pressure, are reported. These studies were made chiefly on groups of patients who showed variations in the activity of the thyroid, pituitary or adrenal glands, as well as a small group of clinical normal controls.

It was found that adrenalin invariably caused an increase in the heat production and in thirty-nine experiments there was an increase in the respiratory quotient. This rise in heat production is accompanied by an increase in the ventilation rate and as a rule by an increase

in the respiration rate, heart beats per minute, volume of each beat, greater utilization of the blood-carrying power and peripheral vascular dilatation with an increased systolic and decreased diastolic blood pressure. Not all these compensatory factors necessarily come into play in each instance. As would be expected, various combinations may occur, sometimes one factor, sometimes another factor acting as the major compensation. The data show that there is no relationship between the character of the adrenalin reaction and the degree of activity of the thyroid gland and, therefore, in our opinion, contrary to that of Goetsch, the reaction is not indicative of the presence or absence of hyperthyroidism.

The similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk from a carbohydrate plethora naturally directs attention to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. There may be, however, in addition a direct chemical stimulation of cellular combustion.

*Further experiments on the effects of warming and cooling the sino-auricular node in the mammalian heart.* BENJ. H. SCHLOMOVITZ.

Dogs and cats were used. Tracings were taken by tambours connected with auricle and ventricle, and time with hundredths-seconds tuning fork. The cardiac tissue, mainly sino-auricular node was warmed or cooled by a special thermode.<sup>1</sup> The results are summarized.

1. The primary cardiac pacemaker maintains its normal locus in both light and deeply etherized animals even to the toxic stage of etherization with auricular dilatation, whether atropine has been given or not. The sino-auricular node responds to both warming and cooling.

2. A persistent tachycardia cannot be produced by warming the sino-auricular node for long periods in lightly anesthetized animals, whether atropinized or not. Instead the rate slows after a transitory tachycardia to a rate close to or less than normal, and upon removal of the thermode the rate usually drops below normal. The A.V. interval may show no change throughout or after the period of warming, or may decrease 0.02 to 0.03 second gradually, or show no consistent variation.

3. An experimental tachycardia of nomotopic origin with short periods of sudden onset and offset resembling certain clinical cases may be produced. Occasionally, an experimental auricular flutter can be shown.

4. In some cases of advanced digitalization of the heart, when idioventricular rhythm prevails, repeated production of ventricular fibrillation following ventricular tachycardia can be instituted by warming any localized portion of the ventricles. This quickly passes off on removal of the thermode.

<sup>1</sup> Schlomovitz and Chase: Arch. Int. Med., 1917, xx, 613.

*Effect of glutamine production on urinary nitrogen.* CARL P. SHERWIN,  
W. WOLF and M. WOLF.

Two men were brought into a condition of minimal nitrogen metabolism by a diet of potatoes, bananas, small amounts of bread, butter and cocoa.

Each man was fed 2.5, 5, 7.5 and 10 grams of phenylacetic acid as a water solution of the sodium salt. Subject no. 1 ingested the acid in single doses while subject no. 2 ingested the acid as 0.5 gram doses at hourly intervals. The phenylacetylglutamine excreted in the urine after the ingestion of the phenylacetic acid was determined quantitatively. Quantitative determinations were also made of total nitrogen, urea, uric acid, creatinine and ammonia.

In subject no. 1 there was an increase in total urinary nitrogen after the ingestion of the acid and a great decrease in the amount of urea nitrogen, a slight increase in ammonia and little or no alteration in the amount of creatinine and uric acid. Subject no. 2 showed a much greater increase in total urinary nitrogen but less alteration in the excretion of urinary nitrogen. Glutamine is produced by the organism in much greater quantities than found in the food. The method of its production is still as uncertain as that of glycocoll.

*The rôle of catalase in the animal organism.* W. E. BURGE.

Substances such as amino acids, sodium salts of the fatty acids, glycerine and sugar were found to produce an increase in catalase when introduced into the alimentary tract by stimulating the liver to an increased output of this enzyme. When an Eck fistula was established these substances produced no increase in catalase. Substances such as the narcotics which produce a decrease in oxidation were found to produce a decrease in catalase by diminishing its output from the liver and by the direct destruction of the enzyme.

The introduction of chlorine into the methane molecule is known to enhance its effect as a narcotic. It was found that it rendered it more effective in decreasing catalase. The introduction of sodium acetate into the alimentary tract stimulates the liver to an increased output of catalase. The introduction of chlorine into the sodium acetate molecule decreases its stimulating effect on catalase production. The tri-chlorine substitution product of acetaldehyde, chloral, decreases catalase more than does acetaldehyde, a weaker narcotic.

*The respiratory quotient and its uncertainty.* J. A. FRIES.

If the term *respiratory quotient* when applied to animals refers to the collective exchange of gases due to the metabolic activity of the individual cells forming the body tissues, then a term is needed which will convey that idea.

Because of its great fluctuations during the day the average of a few short period tests does not always represent the daily respiratory quotient. Further the presence of gases of uncertain origin and quantity, such as fermentation gases of the intestines, is also a source of



much uncertainty. With a fasting man in a respiration calorimeter Benedict obtained closely agreeing daily respiratory quotients, average for 7 days being 0.75 and for 3 days with food an average of 0.817. The difference, however, between the 2-hour periods and the average for the same day was as high as 37.18 per cent. In Carpenter's work the same thing is noticed. In very many instances 15-minute tests, not far apart, varied from 9 per cent to 31.4 per cent from each other.

Likewise with the pulmonary respiratory quotient of oxen (Klein), short tests following closely upon each other varied up to 14.3 per cent and the averages of 7 groups of tests as much as 23.2 per cent. Hence duration of tests and volume of accidental gases are deciding factors in a determination.

Markoff gives ratio of  $\text{CH}_4$  to  $\text{CO}_2$  taken directly from the paunch as 1:3.68 and as 1:5.19 before any absorption takes place. Klein, with oxen during six 24-hour Regnault-Reiset tests, found the daily respiratory quotient = 1.034 and later a pulmonary quotient = 0.882. Mollgaard and Anderson with a milking cow found the pulmonary quotient = 0.967. The writer, with a different method, for milking cows found, using the 1:3.68 and 1:5.19 ratios for  $\text{CH}_4$  to  $\text{CO}_2$ , for cow I, 1.031 and 0.816, and for cow II, 1.078 and 0.856 respiratory quotients respectively.

Methods: Klein used short periods, small samples and pyrogallate; Mollgaard and Anderson long periods, large samples and their hydrogen combustion method; Fries long periods, large samples and his carbon combustion method for oxygen determination.

The present problems are to determine more accurately the ratio of  $\text{CH}_4$  to  $\text{CO}_2$  produced in the intestinal tract and the amount of these gases passing out through the lungs.

*The action of prostatic extracts on isolated genito-urinary organs.* D. I. MACHT and S. MATSUMOTO.

The contractions and tonicity of various surviving excised genito-urinary organs were studied in vitro: first, under normal conditions, and second, after the addition of prostatic extracts to the medium in which the tissues were suspended. The following organs were examined: Uterus and Fallopian tube, bladder and ureters, and vas deferens and seminal vesicle. Aqueous saline extracts of the ram's, dog's, bull's, steer's and human prostate glands were used. It was found that all of the above organs are stimulated in vitro by prostatic extracts, provided a sufficient dose is used; but that different organs require different doses of the glandular extract. The uterus and tubes were found to respond to the smallest quantities of prostatic extract; the bladder and ureters came next in the order of their response to such treatment; while the vas deferens and seminal vesicles required the largest doses of the extracts to give evidence of any physiological effect. As a result of the experiments, the authors conclude that the prostatic extracts cannot be regarded as having any specific or marked influence on the tonus and contractions of the bladder in vitro. Fuller data will appear in due time in the Journal of Urology.



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## CONTENTS

PAGE

BLOOD VOLUME STUDIES. I. EXPERIMENTAL CONTROL OF A DYE BLOOD VOLUME METHOD. <i>C. W. Hooper, H. P. Smith, A. E. Belt and G. H. Whipple</i> .....	205
BLOOD VOLUME STUDIES. II. REPEATED DETERMINATION OF BLOOD VOLUME AT SHORT INTERVALS BY MEANS OF THE DYE METHOD. <i>H. P. Smith</i> .....	221
BLOOD VOLUME STUDIES. III. BEHAVIOR OF LARGE SERIES OF DYES INTRODUCED INTO THE CIRCULATING BLOOD. <i>A. B. Dawson, H. M. Evans and G. H. Whipple</i> .....	232
BLOOD VOLUME STUDIES. IV. BLOOD VOLUME AS DETERMINED BY THE CHANGE IN REFRACTIVITY OF THE SERUM NON-PROTEIN FRACTION AFTER INJECTION OF CERTAIN COLLOIDS INTO THE CIRCULATION. <i>Irvine McQuarrie and Nelson C. Davis</i> .....	257
THE INFLUENCE OF SPLENIC EXTRACT ON THE NUMBER OF CORPUSCLES IN THE CIRCULATING BLOOD. <i>Ardrey W. Downs and Nathan B. Eddy</i> .....	279
PHYSIOLOGIC CHANGES PRODUCED BY VARIATIONS IN LUNG DISTENTION. II. EFFICIENCY OF THE PULMONARY CIRCULATION IN OVERCOMING OBSTRUCTION. <i>Felix P. Chillingworth and Ralph Hopkins</i> .....	289
EXPERIMENTS ON THE PATHOLOGICAL PHYSIOLOGY OF ACUTE PHOSGENE POISONING. <i>Walter J. Meek and J. A. E. Eyster</i> .....	303
TO WHAT EXTENT ARE THE PHYSIOLOGICAL EFFECTS OF CARBON DIOXIDE DUE TO HYDROGEN IONS? <i>M. H. Jacobs</i> .....	321
THE GASTRIC RESPONSE TO FOODS. VII. THE RESPONSE OF THE NORMAL HUMAN STOMACH TO VEGETABLES PREPARED IN DIFFERENT WAYS. <i>Raymond J. Miller, Harry L. Fowler, Olaf Bergeim, Martin E. Reh fuss and Philip B. Hawk</i> .....	332
FURTHER OBSERVATIONS ON THE RELATION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPERGLYCEMIAS (ETHER AND ASPHYXIA). <i>G. N. Stewart and J. M. Rogoff</i> .....	366
THE EXPERIMENTAL PRODUCTION OF EDEMA AS RELATED TO PROTEIN DEFICIENCY. <i>Emma A. Kohman</i> .....	378

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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## BLOOD VOLUME STUDIES

### I. EXPERIMENTAL CONTROL OF A DYE BLOOD VOLUME METHOD

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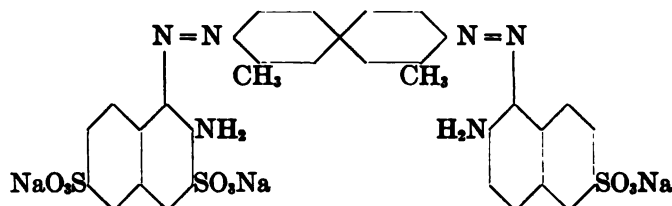
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Studies of blood volume developed in this laboratory as the result of work which was being done in the field of pigment metabolism. Both bile pigment and blood pigment were being studied with particular regard to reciprocal pigment production and destruction in the bile and blood respectively (1). It is obvious that no comprehensive study of blood pigment can be made without reasonably accurate determinations of blood volume. It is remarkable how much effort has been expended in a study of cell counts and hematocrit readings under various experimental conditions with no knowledge concerning the blood volume figures. If a critic chooses to assume any fluctuations in blood volume, all the carefully drawn hypotheses in such papers must become valueless. That such fluctuations in blood volume do occur following a variety of experimental conditions will be shown in experiments to be published in this series of papers.

The final blood volume method as outlined in this paper is the end result of a series of modifications made in the original dye method published by Keith, Rowntree and Geraghty (2). Vital red, the dye used in their experiments, was not obtainable in sufficient quantity and it was necessary to substitute the dye "brilliant vital red." Dr. Herbert M. Evans, Professor of Anatomy, with his expert knowledge of dyes, when consulted concerning the use of other dyes in the estimation of plasma volume, suggested "brilliant vital red." This opportunity is taken to express appreciation of Doctor Evans' interest in

this work and the valuable assistance and materials which he has given so generously.

"Brilliant vital red" is tolidine combined with one molecule of  $\beta$  naphthylamine, 3,6 disulfonic acid and one molecule of  $\beta$  naphthylamine 6 monosulfonic acid.



It is readily soluble in water and does not dialyze against water through celloidin sacs. It is harmless when injected intravenously in the amounts used for the estimation of plasma volume. In none of the many experiments with the blood volume method which have been performed by us have any symptoms been noted that could be attributed to toxicity on the part of the dye. To further test this point 20 cc. of a 1 per cent solution of brilliant vital red were injected into the jugular vein of a mongrel bull pup weighing 6.6 kilos. Although this is approximately fifteen times the amount injected in a blood volume determination no evil effects were observed except a rise of  $0.7^{\circ}\text{C}$ . in temperature for a period of two hours. There is no evidence that the dye has any hemolytic action on normal dog's blood.

#### METHOD

Normal dogs were used in all these experiments. A hypodermic needle is inserted into the vein and 10 cc. of blood drawn (with as little compression of the vein as possible) into a dry well-vaselined syringe. The blood is immediately placed in a 15 cc. graduated hematocrit tube containing 2 cc. of a 1.6 per cent solution of sodium oxalate. The blood and oxalate are mixed by inversion and the tube stoppered. A standard amount of the dye is drawn up into a syringe along with 5 to 10 cc. of 0.9 per cent saline. This dye solution is now injected into the jugular vein, the dye being washed completely out of the syringe into the blood stream by means of a few cubic centimeters of saline. The dye solution is 1 per cent strength and is given in the amount of 1 cc. per 5 kilos body weight. This is the amount arbitrarily taken by us but more or less may be used to suit individual taste in color readings.



Exactly four minutes after the injection of this dye a clean needle is again inserted into the jugular vein (preferably of the opposite side) and another 10 cc. sample of blood drawn and placed in another hematocrit tube also containing 2 cc. of 1.6 per cent sodium oxalate. The hematocrit tubes are now centrifugalized at 2500 revolutions a minute for 30 minutes. The total contents of the tube and the number of cubic centimeters of blood cells are now noted. Two cubic centimeters of the dye-colored plasma are pipetted off and diluted in a small tube with 4 cc. of 0.9 per cent sodium chloride. This unknown is then read in a colorimeter against a standard prepared as follows:

1. Seventy-five-hundredths cubic centimeters of 1 per cent brilliant red vital is pipetted into a 200 cc. volumetric flask which is then made up to the mark with distilled water.

2. Five cubic centimeters of this aqueous dye solution are then diluted with 5 cc. of clear dye-free plasma (obtained from the first sample of blood drawn from the animal) and 5 cc. of 0.9 per cent sodium chloride, making in all 15 cc. of standard dye solution.

Against this standard the above unknown is read and expressed in per cent. This value will henceforth be referred to as *R*.

Let us assign the following values to be used in the formula below:

*D* equals the number of cubic centimeters of 1 per cent brilliant vital red injected into the animal.

*C*, the correction for the dilution by oxalate present equals the total number of cubic centimeters of oxalated plasma present in the second sample of blood drawn, minus 2 divided by the total number of cubic centimeters of oxalated plasma present in the same tube. This value expresses the ratio between the actual concentration of dye in the plasma when diluted with oxalate solution to the value when not so diluted.

*R* equals the observed colorimetric reading (in per cent of the standard).

Plasma per cent means the percentage of the whole blood which the plasma constitutes, and is obtained by dividing the total number of cubic centimeters of oxalated plasma present in the hematocrit tube minus 2, by the total contents of the tube in cubic centimeters minus 2.

Then

$$\text{The plasma volume (in cc.)} = \frac{26666.67 D C}{R}$$

$$\text{The blood volume} = \frac{\text{Plasma Volume} \times 100}{\text{Plasma Per Cent}}$$

The above formula for plasma volume may be derived as follows: The standard for color comparison contains 0.75 cc. of 1 per cent dye in 200 cc. of fluid, or 1 cc. in 266.66+ cc.  $D$  cc. of 1 per cent dye (the amount injected) will impart the same color intensity to 266.6667  $D$  cc. of fluid.

If, however, the color intensity is  $\frac{R}{100 C}$ , (i.e., the colorimetric reading in per cent corrected for the dilution of the plasma by the oxalate solution), the number of cubic centimeters of fluids equals  $\frac{(266.6667 D) (100 C)}{R}$   
 or  $\frac{26666.67 D C}{R}$

#### METHOD CONTROLS

In the method as outlined above the blood drawn from the animal is mixed with a known amount of an isotonic solution of sodium oxalate (see below) instead of with solid sodium oxalate as was done by Keith, Rowntree and Geraghty (2). The experiments cited in table 1 illustrate the fact that shrinking of blood cells results when even small amounts of solid sodium oxalate are added. Thus the minimal amount of oxalate which can be used (10 mgm. to 10 cc. of blood) causes on an average a shrinkage of about 3 per cent in the cell volume. In actual practice a considerable excess of oxalate may be added unless carefully weighed out. In such cases the error is still larger and may in fact be several times this size.

*To obtain the true hematocrit value the anticoagulant used must be isotonic with blood.* It is true that samples of blood drawn from different individuals differ from each other in tonicity, but the variation is very slight except in certain pathological conditions such as uremia. The tonicity of normal blood is generally stated to be equal to that of a 0.95 per cent solution of sodium chloride. As far as we have been able to learn, no accurate physical-chemical investigations have been made to determine the concentration of sodium oxalate isotonic with physiological sodium chloride. Assuming that the dissociations of sodium and potassium oxalates are not very far different we find by calculation from the tables of Noyes and Johnston (3) that a 1.6 or 1.7 per cent solution of sodium oxalate would be approximately isotonic with a 0.95 per cent solution of sodium chloride. Valuable advice covering this physical-chemical work was kindly rendered us by Dr. Carl L. A. Schmidt of the Department of Biochemistry.

A number of experiments have been carried out to determine by *biological tests* that concentration of sodium oxalate which, placed in contact with normal red blood cells, produces neither shrinking nor swelling of these cells. Table 2 below shows the results of two experiments on defibrinated normal dog's blood. In both cases the 1.6 per cent solution of sodium oxalate is approximately isotonic. The effect of oxalate solutions on the blood cells of peptonized blood is given in the third column of the same table. 1.4 per cent is obviously hypotonic, while 1.7 per cent is hypertonic. By interpolation the point of isotonicity is found to be about 1.5 per cent. We may, then, from the physical-chemical data supported by biological experiments, conclude that 1.6 per cent sodium oxalate is approximately isotonic with normal dog's blood.

*Experiment 222. Tonicity of solutions of sodium oxalate*

Two hundred and thirty cubic centimeters of blood were rapidly aspirated from the jugular vein of a normal 40-pound dog (17-160). This blood was immediately defibrinated by whipping.

Two cubic centimeters of sodium oxalate solutions of varying concentrations were pipetted into each of a series of seven tubes. The eighth was left empty. A second and identical series was set up as duplicates. Into each of these tubes were pipetted 9 to 10 cc. of the well-mixed defibrinated blood. The contents of the tubes were mixed, the tubes corked and centrifugalized for one-half hour at 3000 revolutions per minute. The quantity of packed cells and of supernatant fluid was read off on each of the tubes. After making proper allowance for the quantity of fluid added along with the oxalate, the per cent of cells was calculated. The averages for the duplicates are given in table 2.

Identically the same procedure was carried out on a similar quantity of blood obtained from another normal 42-pound dog (621). The results are given also in table 2.

*Experiment 224. Tonicity of solutions of sodium oxalate*

Three grams of "Witte's Peptone" dissolved in 20 cc. of Locke's solution were injected rapidly into the jugular vein of a normal young 10-pound female terrier under complete ether anesthesia. After 30 minutes a cannula was placed in the carotid artery and samples of blood were drawn into each of a series of four hematocrit tubes. Of these tubes three contained each 2 cc. of solutions of sodium oxalate of varying concentration. The fourth tube contained no oxalate and was prevented from clotting by the familiar "peptone reaction." This experiment supplements the data furnished in the two defibrination experiments. All of the tubes were then centrifugalized simultaneously for 30 minutes at 3000 revolutions per minute. The quantity of packed cells and of supernatant fluid was read off on each of the tubes. After making proper allowance for the quantity of fluid added along with the oxalate, the per cent of cells was calculated. The results are given in table 2.

*Dye removed by blood coagulation.* When the error due to the use of dry powdered oxalate was first appreciated we turned to the use of blood serum obtained by coagulation in a vaselined tube. The blood was obtained as usual four minutes after dye injection, allowed to clot

TABLE 1

*Red blood cell hematocrit modified by solid sodium oxalate*

DOG	QUANTITY OF DEFIBRI- NATED BLOOD	HEMATOCRIT (IN PER CENT RED BLOOD CELLS) ON ADDING SOLID SODIUM OXALATE						
		0 mgm.	10 mgm.	20 mgm.	30 mgm.	50 mgm.	70 mgm.	100 mgm.
	cc.							
19-128	10	57.9	55.0	52.0	49.5	43.2	45.6	42.7
17-160	10	57.5	54.5	53.0	50.0	49.5	46.2	44.0
19-106	10	49.8	46.7	45.7	43.8	44.3	43.4	40.0

A fair approximation of the usual amount of dry powdered oxalate used in routine work may be placed at 40 to 60 mgm. per 10 cc. blood.

TABLE 2

*Tonicity of solutions of sodium oxalate*

PER CENT SODIUM OXALATE USED	HEMATOCRIT BLOOD CELL PER CENT		
	Experiment 222, dog 17-160	Experiment 222, dog 621	Experiment 224, dog 19-107, young adult
1.0	48.0	63.0	63.7
1.1	47.7	62.0	
1.2	47.4	61.3	
1.3	46.6	61.2	
1.4	46.1	59.9	61.1
1.5	46.3	60.4	
1.6	45.2	59.1	
Control with no oxalate	45.1	58.9	59.6
1.7			56.6
	Defibrination of blood	Defibrination of blood	Peptone shock blood incoagu- lable

and the serum collected after centrifugalization. This serum was then compared colorimetrically with a known dye standard. Such steps would simplify the procedure as it would then be unnecessary to allow for dilution by the oxalate solution.

This method has been employed as a routine procedure in a large number of experiments which have been performed in this laboratory. Experiments cited below show that certain dangers are involved. Thus while experimenting with a recently purchased sample of brilliant vital red obtained from an English manufacturer it was found that in the process of clotting a certain amount of dye was removed from solution. This is shown in experiment 219. On an average in the three cases here cited about 7 per cent of the dye was removed from the plasma during the process of clotting. For this reason the blood volume values are too high when the serum is used for the dye reading.

The stock dye which has been used in the previous work was tested in a similar series of experiments by Mrs. F. S. Robscheit in this laboratory. In the case of this dye apparently none of the dye was so removed. In several tests made with this same dye we have confirmed this observation. The routine blood volume work already done in this laboratory with this serum method was all done with the old stock dye and therefore cannot be criticised from this standpoint. It is noteworthy that the dye obtained from the English manufacturer was of a different shade of red from the samples which we had previously used. Furthermore it was decidedly weaker in strength so that it was necessary to use a 2 per cent solution for injection instead of the customary 1 per cent solution.

Whether all samples of brilliant vital red when present in larger amounts (as in some of the experiments cited in the following paper) would be removed in the process of clotting is not as yet known. In view of this uncertainty and in view also of the differences in dyes obtained from different manufacturers we feel that to avoid possible error all samples should be collected in isotonic oxalate solution in the manner already described.

*Experiment 219. The effect of blood clotting on the concentration of dye in the plasma*

Three healthy young adult dogs were used in the following experiment:

Dog 19-38. Female Coach dog. Weight 24.68 pounds.

Dog 19-39. Female mongrel terrier. Weight 24.25 pounds.

Dog 18-92. Male mongrel terrier. Weight 18.75 pounds.

One cubic centimeter of brilliant vital red (English) for each five kilos body weight was injected intravenously. The blood volume determination was carried out in the usual way with the exception that in addition to the ordinary hematocrit sample taken four minutes after the injection of the dye, a 10 cc. sample was also drawn at this time into a dry vaseline-lined heavy-walled test tube and allowed to stand 30 minutes. At the end of this time the clot was

freed from the wall of the tube with a piece of wire. After centrifugalization (30 minutes) the serum was drawn off and diluted with two volumes of 0.9 per cent sodium chloride solution. The dye-colored oxalated plasma was obtained from the hematocrit tube in the same way and was similarly diluted. Both of these diluted dye-containing samples were then read colorimetrically against a standard prepared as follows: 0.5 cc. of the 2 per cent brilliant vital red was accurately brought up to 100 cc. in a volumetric flask; 5 cc. of this mixture were mixed with 5 cc. of dye-free plasma and 5 cc. of 0.9 per cent sodium chloride.

The observed readings and estimations for plasma volume and blood volume for each of the three dogs are given in table 3 below. It will be observed that the samples were read against a standard which is stronger than usual. This must be taken into account in making the plasma volume and blood volume calculations.

TABLE 3

*Experiment #19. The effect of blood clotting on the concentration of dye in the plasma*

SPECIMEN READ AGAINST STANDARD	OBSERVED READ- ING AGAINST STANDARD			ESTIMATED PLASMA VOLUME			HEMATOCRIT (CELLS)			ESTIMATED BLOOD VOLUME		
	Dog 19-38	Dog 19-39	Dog 18-92	Dog 19-38	Dog 19-39	Dog 18-92	Dog 19-38	Dog 19-39	Dog 18-92	Dog 19-38	Dog 19-39	Dog 18-92
	per cent	per cent	per cent	cc.	cc.	cc.	per cent	per cent	per cent	cc.	cc.	cc.
Serum*.....	92	83	64	487	530	533	(52.1)	(60.5)	(48.2)	1017	1342	1029
Oxalated plasma (corrected for the dilution by the oxalate so- lution)	99	91	70	453	485	487	52.1	60.5	48.2	946	1228	940

\*This indicates that blood coagulation removed a definite per cent of this dye from the plasma. For this reason the blood volume values are too high when the serum is used for the dye readings.

*Centrifugalization factors.* In order to determine the minimum length of time that is required to cause by centrifugalization at 2500 revolutions a minute a completion of the process of sedimentation, a number of 15 cc. hematocrit tubes filled with oxalated dog's blood were centrifugalized varying lengths of time. The distance of the bottom of the tube from the center of the circle of rotation equals 27 cm. Twenty minutes were found to be insufficient; however, the packing at the end of thirty minutes was in most cases as complete as at any subsequent time. According to Köppe (4) complete packing of the cells is accompanied by a translucency of the layer of packed cells. Such translucency according to him occurs only if the tubes are centrifugalized at a speed

of not less than 5000 revolutions a minute. The experiments no. 253 and 268 presented below show that if any of the dye-colored plasma remains in the interstices it is so small in amount as to be negligible. Moreover, we have performed experiments which show that packed cells from dye-containing blood when washed with very small amounts of isotonic saline impart to this saline only an exceedingly small trace of dye. For all practical purposes therefore the packing may be considered complete at the end of thirty minutes.

*Experiment 268. The principle of the blood volume method tested in vitro*

A total of 1500 cc. of blood was drawn from the jugular veins of four normal dogs into 75 cc. of 1.6 per cent sodium oxalate. This blood was thoroughly mixed. Nine hundred cubic centimeters were poured into a dry 1000 cc. volumetric flask. Two cubic centimeters of 1 per cent brilliant vital red were added and the flask made up to mark with oxalated blood. The contents of the flask were thoroughly mixed by rotation and inversion for 5 minutes. At the end of this time 14 cc. were quickly pipetted into a dry 15 cc. graduated hematocrit tube. This tube of blood was centrifugalized for 30 minutes at 3000 revolutions per minute. The quantity of packed cells and of supernatant fluid was then read off and the per cent of blood cells calculated to be 53.5 per cent.

Two cubic centimeters of the dye-tinged supernatant fluid were mixed with 4 cc. of 0.9 per cent NaCl and read against a standard prepared as follows: 0.75 cc. of 1 per cent brilliant vital red was accurately brought up to 200 cc. with distilled water in a volumetric flask. Five cubic centimeters of this were mixed with 5 cc. of the clear dye-free oxalated plasma and 5 cc. of the 0.9 per cent saline. Against this standard the above diluted sample of dye-colored plasma reads 117.3 per cent.

With this concentration of dye the number of cubic centimeters of oxalated plasma in the total 1000 cc. of blood would, from the previously given formula, be  $\frac{26666.67 \times 2}{117.3} = 455$ . The actual amount of oxalated plasma present (as indicated by the hematocrit) was 465 cc.

*Experiment 253. Blood volume in vitro*

Three hundred cubic centimeters of dog's blood were drawn into a bottle containing 35 cc. of 1.6 per cent sodium oxalate. This blood was thoroughly mixed. Two hundred cubic centimeters were poured into a dry 250 cc. volumetric flask, 0.455 cc. of 1 per cent brilliant vital red was added and the flask made up to mark with oxalated blood. The contents of the flask were thoroughly mixed by rotation and inversion for 5 minutes. At the end of this time two graduated 15 cc. centrifuge tubes were filled, allowed to stand for several minutes and centrifugalized at 2500 revolutions a minute for 30 minutes. The quantity of packed cells and of supernatant fluid was then read off and the per cent of blood cells calculated to be 53.9 per cent.

Two cubic centimeters of the dye-tinged supernatant fluid were mixed with 4 cc. of 0.9 per cent NaCl and read against a standard prepared as follows: 0.75 cc. of 1 per cent brilliant vital red was accurately brought up to 200 cc. with distilled water in a volumetric flask. Five cubic centimeters of this were mixed with 5 cc. of the clear dye-free oxalated plasma (obtained by centrifugalization) and 5 cc. of the 0.9 per cent NaCl. Against this standard the above diluted sample of dye-colored plasma read 100 per cent.

With this concentration of dye the number of cubic centimeters of oxalated plasma in the total 250 cc. of oxalated blood would, from the previously given formula, be  $\frac{26666.67 \times 0.455}{100} = 121$ . The actual amount present, as indicated by the hematocrit, was 115 cc.

*Blood volume method tested in vitro.* The dye method for the determination of blood volume has been tested *in vitro* on large quantities of oxalated

TABLE 4  
Rate of elimination of brilliant vital red from circulation

BLOOD FROM DOG NUMBER	ACTUALLY OBSERVED COLORIMETRIC READING OF THE TWO MINUTE SAMPLE CORRECTED FOR OXALATE	RELATIVE CONCENTRATION OF DYE IN PLASMA AFTER INJECTION OF DYE (TWO MINUTE SAMPLE TAKEN AS 100)				
		Two min- utes after injection	Four min- utes after injection	Twenty min- utes after injection	Three hours after injection	Twenty-four hours after injection
18-39	103.6	100	99.2	93.9		10 to 15
18-28	122.2	100	99.3	89.0	62.4	10 to 15
19-123	136.0	100	102.6	93.0	64.0	10 to 15
19-84	138.3	100	98.3	91.1	59.8	10 to 15
Average..	125.0	100	99.9	91.8	62.1	10 to 15

dog's blood in the experiments given above (268 and 253). The theoretical and the estimated quantity of oxalated plasma in the flask in one case differ by about 5 per cent; in the other by less than 2 per cent. The dye therefore is not absorbed to any appreciable extent by the blood cells nor is it to be found to any appreciable extent in the interstices between the blood cells when the latter are packed by the degree of centrifugalization employed. Furthermore, experiments have shown that no appreciable amount of dye could be extracted from this layer of packed cells by washing with small amounts of physiological saline.

*Elimination of the dye from circulation.* Any blood volume method based on the dilution of a substance introduced into the blood stream depends for its accuracy on a slow rate of elimination from circulation. The rate of elimination of brilliant vital red is shown in table 4. In this table are summarized experiments on four normal active young adult



dogs. An amount of dye equal to that customarily used in blood volume estimation was injected into each dog in the customary way. Ten cubic centimeter samples were collected at intervals after the injection of the dye. As can be seen from table 4, the concentration of the dye in the plasma in most cases is the same at the end of two minutes as at

TABLE 5

EXPERIMENT NUMBER	SEX	DESCRIPTION	WEIGHT	BLOOD VOLUME	PLASMA VOLUME	TOTAL BLOOD CELL VOLUME	HEMATOCRIT CELLS	BLOOD PER 100 GRAM BODY WEIGHT
			kgm.				per cent	cc.
305			7.27	640	310	330	51.6	8.80
233	f	Fox	8.52	892	365	527	59.1	10.47
237	m	Fox	9.09	1041	478	563	54.1	11.45
230	f	Coach	10.97	1197	601	596	49.8	10.91
235	f	Mongrel Spitz	11.14	1255	557	698	55.6	11.27
251	m	Mongrel	13.18	1357	643	714	52.6	10.30
257	m	Mongrel	13.18	1293	742	551	42.6	9.81
258	m	Spitz	13.18	1103	502	601	54.5	8.37
234		Mongrel	13.64	1439	525	914	63.5	10.55
261	m	Mongrel	13.64	1225	767	458	37.8	8.98
256	m	Mongrel	13.86	1314	644	670	51.0	9.48
211	m	Setter	15.91	1835	936	899	49.0	11.53
262	m	Bull	16.36	1487	769	718	48.3	9.09
240	f	Bull-terrier	17.73	1867	788	1079	57.8	10.53
241	m	Bull	17.73	1730	865	865	50.0	9.76
249	m	Setter	17.73	1867	958	909	48.7	10.53
247	m	Spaniel	18.41	2080	1029	1051	50.5	11.30
231	m	Shepherd	18.73	1614	1069	545	34.4	8.62
254	m	Shepherd	20.00	1955	919	1036	53.0	9.78
299			20.00	2119	945	1174	55.4	10.60
236	f	Terrier	20.57	2264	840	1424	62.9	11.01
238	m	Mongrel	22.05	2166	1025	1141	53.6	9.82
Average of dogs weighing from 7 to 15 kgm.			11.61	1160	558	602	52.0	10.04
Average of dogs weighing from 15 to 23 kgm.			18.65	1908	922	986	51.2	10.23
Average of all dogs			15.13	1538	740	794	51.6	10.13

the end of four minutes. At the end of twenty minutes, however, about 6 to 11 per cent is lost while in twenty-four hours only about 10 per cent remains. Blood volume estimations based on samples collected at the four-minute interval cannot therefore be invalidated because of the elimination of dye from circulation. It seems probable that the mixing of dye with the plasma is complete at the end of four minutes.

*The blood volume of normal dogs.* Blood volume figures for twenty-two normal dogs, young and adult, maintained on the usual mixed diet are given in table 5. The average for all animals shows 10.13 cc. of blood per 100 grams of body weight. This value corresponds fairly closely to the results of Keith, Rowntree and Geraghty (dye method) (2), and to those of Meek and Gasser (acacia method) (5), but is considerably higher than that obtained by Grehand and Quinquaud (6) with the carbon monoxide method (8.2 cc. per 100 grams body weight), and also that obtained by the method originally employed by Welcker, which depends on washing out or extracting all of the blood and esti-

TABLE 6

*Female mongrel bull dog, weight 22 pounds. Hemorrhage 262 cc. in 3 minutes*

DOG NUMBER	DATE	BLOOD VOL- UME	PLASMA VOL- UME	TOTAL CELL VOLUME	HEMATOCRIT (CELLS)  per cent	TOTAL PRO- TEIN	ALBUMIN	GLOBULIN	NON-PROTEIN
16-160	March 4.....	1006	533	473	47.0				
	March 6 (before hem- orrhage).....					5.7	4.1	1.6	2.5
	March 6 (1 minute after hemorrhage).....					5.6	4.1	1.5	2.5
	March 6 (5 minutes after hemorrhage).....	741	363	378	51.0	5.3	4.1	1.2	2.3
	March 6 (30 minutes after hemorrhage)....				47.0	5.2	4.1	1.1	2.1
	March 7.....				4.7	3.5	1.2	2.3	
	March 9.....	844	591	253	30.0	4.3	3.3	1.0	2.5
	March 15.....					4.9	3.7	1.2	2.5

inating the hemoglobin. By this method about 7.8 cc. of blood per 100 grams body weight was obtained (7). The reasons for the difference between these methods are as yet not entirely clear. This problem is receiving further study in this laboratory.

In this series practically no difference exists in the amount of blood per unit body weight in respect to the size of the animal.

*Hemorrhage experiments.* In a number of animals the effect of acute hemorrhage on blood and plasma volume was studied. The protocols of four such experiments are presented in tables 6, 7, 8 and 9. In all experiments healthy, sound young dogs were used. The first blood volume estimation in each experiment was performed two days

before the day of the hemorrhage. It is recognized that in individual cases the blood volume may change somewhat in the space of two days. In order to minimize this error the animals were kept under as nearly constant conditions as possible throughout the course of the experiment, all animals being kept on a uniform diet of cracker-meal, lard and butter for a period of five days preceding the hemorrhage. Hemorrhage was effected by rapid withdrawal of blood by aspiration through a needle inserted in the external jugular vein. The animals were but slightly depressed and showed but a slight temporary amount of dyspnea as a result of the loss of blood.

TABLE 7

*Black male mongrel pup, weight 20 pounds. Hemorrhage 404 cc. in 3 minutes*

DOG NUMBER	DATE	BLOOD VOL- UME	PLASMA VOL- UME	TOTAL CELL VOLUME	HEMATOCRIT CELLS  per cent	TOTAL PRO- TEIN	ALBUMIN	GLOBULIN	NON-PROTEIN
16-140	March 4.....	1616	711	905	56	5.0			
	March 6 (before hemor- rhage).....					5.4	3.7	1.7	2.5
	March 6 (1 minute after hemorrhage).....					5.2	4.1	1.1	2.1
	March 6 (5 minutes after hemorrhage).....	1130	531	599	53	5.3	3.9	1.4	2.1
	March 6 (30 minutes after hemorrhage)....					5.1	3.7	1.4	2.1
	March 7.....					4.9	3.6	1.3	2.2
	March 9.....	1222	880	342		4.5	2.9	1.6	2.2
	March 15.....					4.7	3.1	1.6	2.2

The effect of the procedure on the blood volume, plasma volume and cell volume is summarized in table 10. The amount of plasma and of cells removed is calculated from the total amount of blood withdrawn on the assumption that the blood withdrawn contained the same percentage of cells as was present in the blood at the time of performing the first blood volume. Although a certain amount of variation exists in individual experiments, the averages show that the fall in both cell volume and in plasma volume is within a few cubic centimeters of the theoretical. In the course of three days, however, the plasma volume has returned to a level slightly above the original level. Due to the decreased cell volume the blood volume is still

TABLE 8

*Female bull coach mongrel, weight 18 pounds. Hemorrhage 244 cc. in 4 minutes*

DOG NUMBER	DATE	BLOOD VOL- UME	PLASMA VOL- UME	TOTAL CELL VOLUME	HEMATOCRIT (CELLS)  per cent	TOTAL PRO- TEIN	ALBUMIN	GLOBULIN	NON-PRO- TEIN
17-157	March 4.....	976	537	439	45				
	March 6 (before hemor- rhage).....					5.7	4.3	1.4	2.1
	March 6 (1 minute after hemorrhage).....					5.4	3.7	1.7	2.1
	March 6 (5 minutes after hemorrhage).....	724	427	297	41	5.0	3.5	1.5	1.8
	March 6 (30 minutes after hemorrhage)....				40	4.9	3.5	1.4	1.8
	March 7.....				5.0	3.9	1.1	1.6	
	March 9.....	818	597	221	27	4.9	3.9	1.0	1.8
	March 15.....				4.9	3.5	1.4	2.1	

TABLE 9

*Male mongrel bull dog, weight 27.5 pounds. Hemorrhage 264 cc. in 2 minutes*

DOG NUMBER	DATE	BLOOD VOL- UME	PLASMA VOL- UME	TOTAL CELL VOLUME	HEMATOCRIT (CELLS)  per cent	TOTAL PRO- TEIN	ALBUMIN	GLOBULIN	NON-PRO- TEIN
17-205	March 4.....	1055	538	517	49				
	March 6 (before hemor- rhage).....					3.4	3.9	1.5	2.7
	March 6 (1 minute after hemorrhage).....					5.8	4.2	1.6	2.1
	March 6 (5 minutes after hemorrhage).....	757	424	333	44	5.3	4.1	1.2	2.3
	March 6 (30 minutes after hemorrhage)....					5.2	3.9	1.3	2.1
	March 7.....					5.2	3.5	1.7	2.3
	March 9.....	840	605	235	28	4.9	3.5	1.2	2.5
	March 15.....				5.0	3.9	1.1	2.1	

below the original figure. The fact that the cell volume is lower at the end of three days than immediately following the hemorrhage is a peculiar and as yet unexplained phenomenon. More data are being collected on this point.

In all of the hemorrhage experiments the serum proteins were estimated by the micro-refractometric method of Robertson (8). A summary of the figures given in detail in the protocols of the individual experiments is presented in table 11. The amount of circulating serum proteins is decreased as a result of hemorrhage by very nearly the theoretical amount. Regeneration, however, has in the course of

TABLE 10  
*The effect of hemorrhage upon blood volume*

DOG NUMBER	WITHDRAWN		BLOOD VOLUME			PLASMA VOLUME			TOTAL CELL VOLUME		
	Of plasma	Of blood cells	Before	Five minutes after	Three days after	Before	Five minutes after	Three days after	Before	Five minutes after	Three days after
	cc.	cc.									
16-160	139	123	1006	741	844	533	363	591	473	378	253
16-140	142	262	1616	1130	1222	711	531	880	905	599	342
17-157	134	110	976	724	818	537	427	597	439	297	221
17-205	135	129	1055	757	840	538	424	605	517	333	235
Average.....	138	156	1164	838	931	580	436	668	583	402	263

TABLE 11  
*The effect of hemorrhage upon the circulating serum proteins*

DOG NUMBER	SERUM PROTEIN WITHDRAWN	CIRCULATING SERUM PROTEINS			SERUM PROTEIN REGENERATED	
		Before hemorrhage	Five minutes after hemorrhage	Three days after hemorrhage	In five minutes	In three days
	grams	grams	grams	grams	grams	grams
16-160	8.07	30.38	19.24	25.41	-3.07	3.10
16-140	9.74	38.39	28.14	41.36	-0.51	12.71
17-157	7.72	30.61	21.35	29.25	-1.54	6.36
17-205	7.91	29.05	22.47	29.65	+1.33	8.51
Average.....	8.36	32.11	22.80	31.42	-0.95	7.67

three days restored the circulating proteins almost to the original figure. It is noteworthy, however, that the concentration of protein in the serum is still considerably below the original.

In conclusion and on the behalf of the Department of Anatomy and the Hooper Foundation we wish to express our appreciation for invaluable assistance given by Dr. Carl Alsberg, Chief of the Bureau

of Chemistry, Department of Agriculture, Washington, and Dr. H. D. Gibbs, Chemist in charge of the Color Laboratory, Bureau of Chemistry, Department of Agriculture, Washington. These gentlemen supplied practically all the dye used in the first two papers of this series.

#### SUMMARY

A simple and accurate method of blood volume determination is outlined in detail. Suitable controls of many factors are submitted and the method tested *in vitro*.

The average normal blood volume for young active dogs of medium weight as determined by this method may be stated as 10.1 per cent body weight. Individual differences are considerable (table 5).

After the rapid withdrawal (hemorrhage) of a known amount of blood, this blood volume method performed immediately will show a *calculated blood volume* remaining which is very close to the *estimated blood volume*.

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## BLOOD VOLUME STUDIES

### II. REPEATED DETERMINATION OF BLOOD VOLUME AT SHORT INTERVALS BY MEANS OF THE DYE METHOD

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A simple method for the estimation of blood volume in the same animal at short intervals is much to be desired. An accurate method for the estimation of blood volume repeatedly at intervals of minutes or hours will at once give data of interest and value. Such data are not at present available. With the ability to estimate blood volume changes during short intervals we are at once able to study the exchange of fluids which may go on in acute shock, or after hemorrhage or induced plethora. Under such conditions one suspects that there may be exchange of fluid between the tissues and the circulating blood but there are no published experiments which are not open to criticism.

Certain observations of this nature have been reported by a few of the earlier workers—Keith, Rowntree and Geraghty (1), Robertson and Bock (2) and Lamson (3), but no details are given as to the exact method used and only one or two observations are recorded in each report. For this reason and because of our earlier experiments we conclude that these observations were not entirely satisfactory.

When one attempts to repeat the blood volume estimation on the same animal at short intervals he is at once confronted by the fact that after one such determination the plasma remains colored for a period varying from several hours to several days. The presence of dye in the plasma complicates all subsequent blood volume determinations. In order to determine the blood volume a second time under such circumstances, one may again inject intravenously the customary amount of dye and estimate colorimetrically the *increase* in dye concentration in the plasma as a result of such injection. Theoretically this increase in color intensity might be estimated by subtracting the colorimetric reading of a sample of plasma drawn before injection of the

dye from the colorimetric reading of another sample taken after such injection. The increase in color intensity thus determined is, however, subject to the error of two colorimetric readings, the sum of which may in some cases be considerable. For example, the second determination may be made after an interval of a few hours. At such time the amount of dye in the plasma may be small and therefore difficult to estimate accurately because of the normal yellow plasma tint. The procedure used to minimize this error is given below. The mathematical soundness of this procedure will be shown.

#### METHOD

For a repeated blood volume estimation the dye is injected and the samples collected in the same way as was outlined in the preceding paper (4). Part of this may for convenience be repeated. A hypodermic needle is inserted into the jugular vein and 10 cc. of blood drawn (with as little compression of the vein as possible) into a dry well-vaselined syringe. This blood is immediately run into a 15 cc. graduated hematocrit tube containing 2 cc. of a 1.6 per cent solution of sodium oxalate. The blood and oxalate are mixed by inversion and the tube stoppered. An amount of brilliant vital red (1 per cent) equal in cubic centimeters to the weight of the animal in pounds divided by 11 (i.e., 1 cc. per 5 kilos body weight) is drawn up into a syringe along with 5 to 10 cc. of 0.9 per cent saline. This dye solution is now injected into the jugular vein, the dye being washed completely out of the syringe into the blood stream by means of a few cubic centimeters of normal saline. Four minutes after the injection of dye a clean needle is again inserted into the jugular vein (preferably of the opposite side) and another 10 cc. sample of blood is drawn and placed in another 15 cc. hematocrit tube also containing 2 cc. of 1.6 per cent sodium oxalate.

The second blood volume determination may be done at such time as may be desired—for example, after a period of 10 or 20 minutes. The procedure is exactly similar to that already described and the time interval of 4 minutes is the same as before, that is, 4 minutes from time of injection of the second dye sample to the time of removal of the second dye plasma sample.

The hematocrit tubes are now centrifugalized at 2500 revolutions a minute for 30 minutes. The total contents of the tube and the number of cubic centimeters of blood cells are now noted. Colorimetric readings are then made and the plasma volume estimated as follows:



*Calculation of the second blood volume*

Let  $D$  = the number of cubic centimeters of 1 per cent dye injected into the animal.

$C_1$  = the total number of cubic centimeters of oxalated plasma present in the first sample of blood drawn minus 2, divided by the total number of cubic centimeters of oxalated plasma present in the same tube. This value expresses the ratio of the actual concentration of dye in the plasma when diluted with oxalate solution, to the value when not so diluted.

$C_2$  = the total number of cubic centimeters of oxalated plasma present in the second sample of blood drawn minus 2, divided by the total number of cubic centimeters of oxalated plasma present in the same tube. The significance of this figure is the same as that described for  $C_1$  above.

$R_1$  = the observed colorimetric reading of a sample of dye-tinged plasma (diluted with two parts of saline) taken immediately before the injection of the dye for the second blood volume estimation read against a standard prepared as follows: Of a dye solution containing 0.75 cc. of 1 per cent dye diluted to 200 cc. with water take 5 cc. To this amount add 5 cc. of 0.9 per cent saline and 5 cc. of normal dye-free plasma.

$R_2$  = the observed colorimetric reading (expressed in per cent) of a sample of dye-colored plasma (diluted with two parts of saline) taken 4 minutes after the injection of the dye for the repeated blood volume estimation, against a standard prepared as follows: 5 cc. of a dye solution containing 0.75 cc. of 1 per cent dye in 200 cc. of water, + 5 cc. of 0.9 per cent saline + 5 cc. of the dye-tinged plasma taken immediately before the injection of dye for the repeated blood volume estimation.

Then:

$$\text{The plasma volume} = \frac{2666666 D C_1 C_2}{C_1 R_2 (R_1 + 100) - 100 C_2 R_1}$$

$$\text{And the blood volume} = \frac{\text{Plasma Volume} \times 100}{\text{Per Cent Plasma}}$$

The total blood-cell volume = blood volume minus plasma volume.

The method above described is essentially the same method as is employed in the estimation of the first blood volume. It will be recalled that in the method as outlined in the previous paper the standard against which the unknown (diluted with two parts of saline) was read, was made up of equal parts of saline, standard dye solution (0.75 cc.

of 1 per cent dye diluted with water to 200 cc.) and normal plasma taken from the same animal immediately before injection of the dye. In performing the second blood volume the unknown and standard are prepared in exactly the same way. In this case, however, the plasma taken immediately before the injection of dye is itself dye-colored (from dye remaining from the previous blood volume determination). This amount of dye ( $R_1$ ) is present in both standard and unknown.

Hence:  $\frac{R_1 + R_2}{R_1 + 100} = \frac{R_2}{100}$  whence  $R_1$  = the colorimetric reading of the plasma before the injection of dye against a standard prepared in the customary way for the first blood volume estimation (equal parts of normal plasma, saline and standard dye solution).  $R_2$  represents the increase in concentration of dye in the plasma as a result of the injection of dye. This formula simply means that the unknown ( $R_1 + R_2$ ) divided by the standard ( $R_1 + 100$ ) equals the observed colorimetric reading expressed in parts per 100 (i.e.  $\frac{R_2}{100}$ ).

The soundness of this last formula is illustrated experimentally in experiment no. 243. In this experiment the standard employed in no. 4 may be taken as 100. The standard used in no. 5 then equals  $100 + R_1$  where  $R_1$  (according to no. 4) equals 75. The unknown in no. 5 also contains  $R_1$  and hence may be represented as  $R_1 + R_2$  where  $R_2$  equals the increase in dye concentration to be determined.  $R_2$ , the observed reading, is 86. By substituting in the formula  $\frac{R_1 + R_2}{R_1 + 100} = \frac{R_2}{100}$  one obtains  $\frac{75 + R_2}{75 + 100} = \frac{86}{100}$  Solving,  $R_2 = 75.5$ .

It can readily be seen from no. 3 that the increase ( $R_2$ ) in dye concentration over the residual is 75. The increase as determined colorimetrically with the use of the formula differs from the correct value, therefore, by only 0.5 per cent.

*Experiment 243. Measurement of the color increase resulting from the addition of dye to dye-tinged water.*

1. Seventy-five one hundredths cubic centimeter of 2 per cent brilliant vital red are accurately diluted with water to 200 cc. in a volumetric flask.
2. Six hundred and seventy-five thousandths cubic centimeter of a 2 per cent solution of the same dye is similarly diluted with water to 240 cc. This is equivalent to diluting 0.56 cc. of dye to 200 cc.
3. Fifty-six hundredths cubic centimeter more of 2 per cent dye is accurately diluted with no. 2 to 200 cc. in a volumetric flask.

4. A standard is prepared as follows: 5 cc. of no. 1 + 10 cc. of water. Against this standard 5 cc. of no. 2 diluted with 10 cc. of water reads 75 per cent.

5. A second standard is prepared as follows: 5 cc. of no. 1 + 5 cc. of no. 2 + 5 cc. of water. Against this standard 5 cc. of no. 3 diluted with 10 cc. of water reads 86 per cent.

It was stated above that mathematically, it would be possible to determine the increase in color intensity by a simple subtraction of the residual dye concentration ( $R_1$ ) from the total dye concentration after addition of more dye. With such procedure it was pointed out that the error in the estimated increase is the algebraic sum of the errors of these two colorimetric readings. Computation shows that by the procedure outlined with the use of the above formula the error in the estimated increase ( $R_2$ ) is not so great as this algebraic sum provided that  $R_1$  is less than  $R_2$ . Moreover as  $R_1$  becomes smaller its accurate determination becomes increasingly difficult particularly since the interference due to the normal pigments of the plasma becomes more marked. Fortunately an error of considerable magnitude may be made in the estimation of  $R_1$  without seriously affecting the value of  $R_2$ . For example if through error in colorimetric reading  $R_1$  in experiment no. 243 had been incorrectly read as 70 instead of 75,  $R_2$  would have been calculated to be 76.2 instead of 75.5—an error of less than 1 per cent.

In all blood volume determinations the plasma is diluted by the oxalate solution which must necessarily be added to prevent coagulation. Correction must therefore be made for such dilution. The formula

$$\frac{R_1 + R_2}{R_1 + 100} = \frac{R_2}{100} \text{ becomes}$$

$$\frac{\frac{R_1 C_2}{C_1} - R_2}{R_1 + 100} = \frac{R_2}{100} \text{ or } R_2 = \frac{R_1 R_2 C_1 + 100 C_1 R_2 - 100 R_1 C_2}{100 C_1}$$

In the previous paper it was found that Plasma Volume =  $\frac{26666.666 D C_2}{R}$ . Since  $R$  in this formula corresponds to the  $R_2$  in the

formula derived above ( $R_2$  being the increase in dye concentration without correction for dilution by oxalate),  $R_2$  may be substituted in the formula for plasma volume with the following result:

$$\text{Plasma Volume} = \frac{26666.667 D C_1 C_2}{C_1 R_2 (R_1 + 100) - 100 C_2 R_1}$$

The experiment cited below (from exper. 218) illustrates the procedure.

*Dog, weight 47.5 pounds.* At 10.45 a.m. a simple blood volume estimation was carried out. Preparations were then made to repeat the determination as has been outlined above. At 11.07 a.m. a sample of blood drawn from the jugular vein was placed in a 15 cc. hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate. After centrifugalization this tube was found to contain 5.95 cc. of blood cells. Its total content was 14.75 cc.  $C_1$  estimated as described above is  $\frac{68}{88}$ . At 11.08 a.m. brilliant vital red (4.30 cc. of 1 per cent solution) together with about 6 cc. of 0.9 per cent sodium chloride were injected into the jugular vein. At 11.12 a.m. another sample of blood was drawn into another hematocrit tube containing also 2 cc. of 1.6 per cent sodium oxalate. After centrifugalization this tube was found to contain 5.6 cc. of blood cells. Its total content was 14.6 cc.  $C_2$  therefore is  $\frac{70}{90}$ .

One cubic centimeter of plasma from the blood sample drawn at 11.07 a.m. was mixed in a small tube with 2 cc. of 0.9 per cent sodium chloride. This solution was read against a standard prepared as follows: 5 cc. of a dye solution containing 0.75 cc. of 1 per cent brilliant vital red diluted to 200 cc. with water + 5 cc. of 0.9 per cent sodium chloride + 5 cc. of normal plasma (obtained from the same animal at 10.43 a.m., i.e., before he had received the first injection of dye). The test solution reads against this standard 76 per cent.  $R_1$  therefore = 76.

A second standard, this time containing dye-tinged plasma, was prepared as follows: 5 cc. of plasma from the sample of blood drawn at 11.07 a.m. + 5 cc. of a dye solution containing 0.75 cc. of 1 per cent brilliant vital red diluted to 200 cc. with water + 5 cc. of 0.9 per cent sodium chloride. A test solution was prepared by diluting 1 cc. of plasma obtained from the sample of blood drawn at 11.12 a.m. with 2 cc. of 0.9 per cent saline. This solution against the above standard reads 90 per cent.  $R_2$  therefore = 90.

Substituting these values in the formula:

$$\text{Plasma Volume} = \frac{2666667 D C_1 C_2}{C_1 R_2 (R_1 + 100) - 100 C_2 R_1}$$

we have

$$\text{Plasma Volume} = \frac{(2666667) (4.30) \left(\frac{68}{88}\right) \left(\frac{70}{90}\right)}{\left(\frac{68}{88}\right) (90) (76 + 100) - (100) \left(\frac{70}{90}\right) (76)} = 1089 \text{ cc.}$$

The per cent plasma (obtained from an average of the two hematocrit tubes)

is 54.4. Since the blood volume =  $\frac{\text{Plasma Volume} \times 100}{\text{Plasma Per Cent}}$  the blood volume =

$$\frac{1089 \times 100}{54.4} = 2002.$$

By difference the total cell volume is 913 cc.

## DISCUSSION

In tables 1 and 2 the dye method for the repeated estimation of blood volume is tested out *in vitro* on large quantities of oxalated dog's blood. In the experiment cited in table 1 three separate blood volume determinations are made on known amounts of the same sample of oxalated blood. In no case was the error greater than 3.1 per cent while the average error for the three determinations was 2.5 per cent. In the experiment cited in table 2 the errors in the estimation by the dye method were 2.5 and 5.0 per cent respectively in each of two determinations. The mechanical errors involved in the repeated estimations of blood volume by the dye method under such experimental conditions do not exceed 5 per cent.

In the three experiments, tables 3, 4 and 5, repeated blood volume determinations have been made on the same dogs at short intervals of time. In all cases repeated determinations on the same animal give similar results. In no case does any blood volume determination differ from the average of all determinations made on the same animal by more than 5.0 per cent.

We wish to emphasize, however, that these figures apply to determinations completed within two to three hour periods. When longer intervals elapse between blood volumes we may observe fluctuations in blood cell and plasma figures which we cannot at present explain. We feel that sufficient data are submitted to show that these fluctuations are not due to errors inherent in this proposed method. When we consider the various physiological reactions of the blood vessels in response to familiar digestive stimuli we need not be surprised at fluctuations in circulating blood volume. These physiological reactions are merely suggested as a type of the many changes which might modify the fluid or cells circulation in the body at any given time.

*Experiment 268. The repeated blood volume method carried out in vitro on oxalated blood.*

1. Seven hundred and fifty cubic centimeters of blood were drawn from the jugular veins of each of four normal dogs into each of two bottles containing 75 cc. each of 1.6 per cent sodium oxalate. This blood was thoroughly mixed. A graduated 15 cc. hematocrit tube was filled.

2. Nine hundred cubic centimeters of no. 1 were poured into a dry 1000 cc. volumetric flask. Two cubic centimeters of 1 per cent brilliant vital red were added and the flask made up to mark with no. 1. The contents of the flask were thoroughly mixed by rotation and inversion for 5 minutes. At the end of this time 14 cc. were quickly pipetted into a dry 15 cc. graduated hematocrit tube.

3. Five hundred cubic centimeters of no. 2 were poured into a volumetric flask calibrated to hold 510 cc. One and two hundredths cubic centimeter of 1 per cent brilliant vital red were added and the flask made up to mark with no. 2. The contents of the flask were thoroughly mixed by rotation and inversion for 5 minutes. At the end of this time a dry graduated 15 cc. hematocrit tube was filled with the mixture.

4. Four hundred and eighty-five cubic centimeters of no. 3 were poured into a volumetric flask calibrated to hold 490 cc. Ninety-eight hundredths cubic centimeter of a 1 per cent brilliant vital red were added and the flask made up to mark with no. 3. The contents of the flask were thoroughly mixed by rotation and inversion for 5 minutes. At the end of this time a dry graduated 15 cc. hematocrit tube was filled with the mixture.

All of the hematocrit tubes were corked and centrifugalized for 30 minutes at 2500 revolutions a minute. The quantity of packed cells and of supernatant fluid in each tube was noted.

The standard solutions and dilutions employed in obtaining the colorimetric readings given below were made in the usual manner except that the aqueous dye solution from which the standards were prepared contained 1 cc. of dye diluted to 200 cc. with water instead of the customary 0.75 dye diluted to 200 cc. with water.

In the first volume estimation (no. 2 above)  $R_2 = 88$  per cent.  $R_1$  of course in this case is zero.

In the second volume estimation (no. 3 above)  $R_2 = 91$  per cent. From the preceding paragraph it is obvious that  $R_1 = 88$  per cent.

In the third volume estimation (no. 4 above)  $R_2$  (no. 4 above) = 94 per cent.  $R_1$  (estimated from the sample prepared in no. 3 above) is 170 per cent.

Since the hematocrit tubes receiving the blood contained no oxalate solution,  $C_1$  and  $C_2$  in all cases equal unity. The final results must be multiplied by  $\frac{1}{2}$  because of the stronger standard used.

The results of the three volume estimations are given in the table below.

*Experiment 253. The repeated blood volume method carried out in vitro on oxalated blood.*

1. Three hundred cubic centimeters of blood were drawn from a normal dog into a flask containing 35 cc. of 1.6 per cent sodium oxalate. This blood was thoroughly mixed. A graduated 15 cc. hematocrit tube was filled.

2. Two hundred cubic centimeters of no. 1 were poured into a dry 250 cc. volumetric flask. Then 0.455 cc. of 1 per cent brilliant vital red was added and the flask made up to mark with no. 1. The contents of the flask were thoroughly mixed by rotation and inversion for 5 minutes. At the end of this time 14 cc. were quickly pipetted into a dry 15 cc. hematocrit tube.

3. One hundred and fifty cubic centimeters of no. 2 were then poured into a volumetric flask calibrated to hold 200 cc. Then 0.356 cc. of 1 per cent brilliant vital red was added and the flask made up to mark with no. 2. The contents of the flask were thoroughly mixed by rotation and inversion for five minutes. At the end of this time a dry graduated 15 cc. hematocrit tube was filled with the mixture. All of the hematocrit tubes were corked and centrifugalized for 30 minutes at 2500 revolutions a minute. The quantity of packed cells and of supernatant fluid in each tube was noted.

TABLE 1

*Experiment #68. The repeated blood volume method carried out in vitro on oxalated blood*

VOLUME DETERMINATION	VOLUME OF OXALATED PLASMA		HEMATOCRIT (CELLS)	VOLUME OF OXALATED BLOOD			TOTAL VOLUME OF PACKED CELLS	
	Actual (by hematocrit)	Estimated by dye method		Actual	Estimated by dye method	Error	Actual (by hematocrit)	Estimated by dye method
	cc.	cc.	per cent	cc.	cc.	per cent	cc.	cc.
First.....	465	454	53.5	1000	976	2.4	535	522
Second.....	271	246	53.2	510	528	3.1	266	280
Third.....	261	234	53.2	490	500	2.0	261	266

The standard solutions and dilutions employed in obtaining the colorimetric readings given below were made in the usual manner.

In the first volume estimation (in no. 2 above)  $R_1 = 100$ .  $R_1$  of course in this case is zero.

In the second volume estimation (no. 3 above)  $R_2 = 100$ . From the preceding paragraph it is obvious that  $R_1 = 100$  per cent.

Since the hematocrit tubes receiving the blood contained no oxalate solution,  $C_1$  and  $C_2$  in all cases equal unity.

The results of the two volume estimations are given in table 2 below.

TABLE 2

*Experiment #63. The repeated blood volume method carried out in vitro on oxalated blood*

VOLUME DETERMINATION	VOLUME OF OXALATED PLASMA		HEMATOCRIT (CELLS)	VOLUME OF OXALATED BLOOD			TOTAL VOLUME OF PACKED CELLS	
	Actual (by hematocrit)	Estimated by dye method		Actual	Estimated by dye method	Error	Actual (by hematocrit)	Estimated by dye method
	cc.	cc.	per cent	cc.	cc.	per cent	cc.	cc.
First.....	115	121	53.9	250	262	5.0	135	141
Second.....	92	95	53.9	200	206	3.0	108	111

*Experiment #18. Repeated determination of blood volume on the same animal. Dog 19-117. Short haired, adult male bull dog. Good condition. Weight 47.5 pounds. Blood volume determinations were carried out according to the procedure already described and at the intervals indicated below:*

First blood volume determination made at 10.43 a.m.

Second blood volume determination made at 11.08 a.m.

Third blood volume determination made at 11.27 a.m.

Due to the taking of blood samples for other purposes a larger amount of blood was withdrawn than is customary—a total of 108 cc.

The results of the determinations are given in table 3 below.

TABLE 3

*Experiment 218. Repeated determination of blood volume on the same animal*

VOLUME DETERMINATION	TIME	PLASMA VOLUME	HEMATOCRIT (CELLS)	TOTAL BLOOD VOLUME	TOTAL VOLUME OF CELLS
		cc.	per cent	cc.	cc.
First.....	10.43	1048	47.5	1994	946
Second.....	11.08	1074	45.6	1972	898
Third.....	11.27	1088	44.1	1946	858
Average.....		1070	45.7	1971	901

*Experiment 227. Repeated determination of blood volume on the same animal. Dog 17-160. Short haired, brown adult male mongrel. Good condition. Weight 45 pounds. Blood volume determinations were carried out according to the procedure already described and at the intervals indicated below:*

First blood volume determination made at 9.58 a.m.

Second blood volume determination made at 10.15 a.m.

The total amount removed was 45 cc.

The results of the determinations are given in table 4 below.

TABLE 4

*Experiment 227. Repeated determination of blood volume on the same animal*

VOLUME DETERMINATION	TIME	PLASMA VOLUME	HEMATOCRIT (CELLS)	TOTAL BLOOD VOLUME	TOTAL VOLUME OF CELLS
		cc.	per cent	cc.	cc.
First.....	9.58	1478	56.7	3413	1935
Second.....	10.15	1478	57.7	3494	2016
Average.....		1478	57.2	3454	1976

*Experiment 246. Repeated determination of blood volume on the same animal. Dog 19-111. Long haired, adult male shepherd. Good condition. Weight 35 pounds. Blood volume determinations were carried out according to the procedure already described and at the intervals indicated below:*

First blood volume determination made at 10.30 a.m.

Second blood volume determination made at 10.45 a.m.

Third blood volume determination made at 1.55 p.m.

Total amount of blood removed was 69 cc.

The results of the determinations are given in table 5 below.

In conclusion I wish to express appreciation to Dr. G. H. Whipple and to Mr. A. E. Belt for advice given throughout the course of this work and for valuable assistance given in the matter of arrangement and presentation of the subject matter contained in this paper.



TABLE 5

*Experiment 246. Repeated determination of blood volume on the same animal*

VOLUME DETERMINATION	TIME	PLASMA VOLUME	HEMATOCRIT (CELLS)	TOTAL BLOOD VOLUME	TOTAL VOLUME OF CELLS
		cc.	per cent	cc.	cc.
First.....	10.30	909	48.5	1765	856
Second.....	10.45	896	47.1	1694	798
Third.....	1.55	994	45.4	1821	827
Average.....		933	47.0	1760	827

## SUMMARY

A method is outlined in which the dye blood volume method is adapted to repeated determinations on the same animal at short intervals. The soundness of the method is demonstrated by controls done *in vitro* as well as repeated determinations performed in rapid succession on the same animal.

The experimental error does not exceed 5 per cent. Fluctuations in blood volume greater than this are sometimes seen over long periods of time. These fluctuations arise from physiological factors, the exact nature of which is not yet understood.

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## BLOOD VOLUME STUDIES

### III. BEHAVIOR OF LARGE SERIES OF DYES INTRODUCED INTO THE CIRCULATING BLOOD

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Blood volume determinations by means of dye substances injected intravenously are now familiar procedures. A dye suitable for this work must be non-toxic and not permanently stored by the body cells. More important, the dye must remain in the circulating plasma at least for several minutes after its injection before its removal from the plasma is much in evidence. "Vital red" dyes have been used for blood volume determinations in human beings and animals with no untoward effects. Some of the errors which may creep into these determinations have been pointed out in other papers of this series (papers I and II). The method used in these experiments is described in detail below.

In view of the increasing interest and importance of blood volume studies it seemed desirable to study with care many dye substances which might be of value in future work—possibly of greater value than the "vital red" series. This study has been extended to include a number of dyes which are excreted in considerable measure by the kidneys or liver. In many instances the behavior of a certain dye was well known and is included in our tables mainly for comparison and control of other factors. Other dyes were tested because of a suggestive chemical constitution which would seem to indicate a possible physiological relationship to other familiar dyes. Still other dyes of this series were employed because they had been studied as to other aspects of their behavior in living animals by workers in the Department of Anatomy. It need not be repeated that at present *the behavior of a given dye in the blood stream cannot be predicted from any analysis of its chemical formula.*

We have tested by the method described below a series of over sixty dye substances. The dyes are predominantly of the benzidine series, are non-toxic and not chemically changed in the blood plasma. For the sake of convenience in tabulation these dyes have been grouped into three main divisions but it should be kept in mind that there is no sharp line of demarkation. These groups fade into each other. Group I contains dyes resembling the "vital red" series which persist in the blood plasma. Group II contains dyes which leave the blood stream rapidly and appear in the urine, for example, phenolsulphonephthalein. Group II contains dyes which leave the blood stream with considerable rapidity but do not appear to any degree in the urine. What becomes of such substances we are unable to say but it is evident that these dyes are changed by contact with the living cells so that they are no longer recognizable.

From the standpoint of blood volume the dyes of group I are of especial interest, but some significance attaches to the physiological behavior of the dyes in groups II and III. An analysis of the dyes in group I (table 5) will show a blue azo dye (T-1824) which we believe to be slightly superior to the best of the vital red series. This dye gives a blue color to the plasma which is very easy to read and can be colorimetrically determined with great accuracy. It leaves the blood plasma very slowly and hemolysis which may be present as a confusing factor in certain experiments is more easily recognized than is the case with the red dyes. Six other dye substances (table 5) may be classified with the "vital red" group for routine blood volume work and if available may be substituted for vital red.

#### METHOD

Unless otherwise noted a 1 per cent solution of the dye made up in distilled water and filtered is used for injection. Calculated on the basis of 2 mgm. per kilogram of body weight, the proper amount of dye is slowly injected into the jugular vein from a glass syringe. This syringe is then rinsed by withdrawing a little blood which is reinjected and followed by a little saline. Previous to the dye injection, a 10 cc. sample of blood is withdrawn in a clean 10 cc. syringe (rinsed in 0.9 per cent salt solution) and emptied into a 15 cc. graduated hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate solution. The tube is inverted twice to insure thorough mixing. Following this two 10 cc. samples of blood are withdrawn and emptied into two vaselined test

tubes. This provides serum for the standard dye mixture. To determine the rapidity of disappearance of the dye from the blood stream, samples are taken in vaselined test tubes after the injection of the dye at the following intervals and in the amounts indicated: after 2, 6, 10, 25 minutes, one 10 cc. sample; after 4, 15 and 40 minutes, two 10 cc. samples. After stoppering, the tubes are centrifuged at 2500 revolutions per minute for 30 minutes. The hematocrit readings (total volume minus 2 cc. oxalate, total cell volume and volume of red cells) are recorded. The normal serum for the standard is pipetted off into a clean dry test tube. The dyed serum samples are also pipetted off into a series of clean, dry tubes.

*Standard:* One-half cubic centimeter of the 1 per cent dye solution (5 mgm. dye) is made up to 100 cc. with distilled water in a volumetric flask.

Five cubic centimeters of the above dye standard are diluted with 5 cc. normal serum and 5 cc. 0.9 per cent salt solution (dilution 1 in 3). This standard reads 100 per cent.

*Dilution:* Tubes—Dyed plasma mixed with 0.9 per cent salt solution in the proportion 2 cc. to 4 cc. respectively (dilution 1 in 3).

*Colorimetric determinations:* Each of the samples of the diluted dyed serum placed successively in a colorimeter is compared with the standard in the wedge of the colorimeter and the average of five readings is recorded as the color per cent of the sample.

*Computation of results:* From the hematocrit readings per cent plasma and red cells may be easily obtained. It will be recalled that each 40 cc. of the standard contains 2 mgm. of the dye.

$$\text{Plasma Volume in cc.} = \frac{\text{Weight in Kilograms} \times 40}{\text{Average Colorimetric Reading}} \times 100$$

$$\text{Blood Volume in cc.} = \frac{\text{Plasma Volume}}{\text{Plasma Per Cent}} \times 100$$

*Determination of amount of dye in the urine:* The dog is catheterized and the bladder washed out with about 20 cc. of warm water at the beginning of the experiment. Collection of urine by catheter is made one hour after injection of the dye and at hourly intervals thereafter until the amount of dye present is too small for colorimetric determination.

Total amount of urine plus washings from each collection is measured, neutralized and made up to a volume with sufficient water to give a

color slightly less intense than the standard dye solution with which a filtered sample is compared. The volume may be 100 cc. or some multiple of 100.

*Standard:* One-half cubic centimeter of a 1 per cent solution of the dye, made up to 100 cc. volume with distilled water or with diluted neutral urine when specimens are not the same color as the standard aqueous solution. This standard contains 10 mgm. of dye per cubic centimeter. Whenever the first hour sample of urine apparently contains no dye, or only a trace, a portion is made acid and another alkaline to make sure that the dye is not present in some colorless form or compound easily dissociated by acid or alkali.

*Dogs:* For the major part of the work six normal male dogs were selected ranging in weight from 45 to 63 pounds. They were used in sequence each day so that each dog was used but once a week. This arrangement gives time for the dye to leave the plasma between determinations and also prevents the development of anemia from too frequent hemorrhage, as each experiment necessitates the withdrawal of 130 cc. of blood. The dogs are always fed after the experiment. This insures an absence of lipemia which may be a disturbing factor.

*Controls for the method:* For each dye duplicate experiments have been made on different dogs and with many of the dyes three to six determinations were made. Small dogs were used in addition to the large ones giving results which are consistent with those obtained for the standard dogs. Duplicate tests have been made with certain dyes on the same dog with essentially identical results.

Duplicating the blood samples for the 4, 15 and 40 minute periods gives control for the technique employed including the colorimetric readings. As a rule both tubes taken at the same time interval gave identical readings, the exception being a variation of not more than 2 per cent. To obviate the personal factor in making colorimetric readings, all determinations were made by the same observer throughout this series of experiments.

Often the samples and standard have been kept in the ice-box for a second colorimetric comparison on the following day. These late readings were practically identical with those of the freshly prepared specimens. All recorded observations, however, were made on the same day of the experiment.

*Experiments using large amounts of dyes:* By injecting 20 mgm. per kilogram of body weight, curves showing the rate of disappearance of dyes from the plasma have been worked out for brilliant vital red

(new); T-1824; T-1835 (alkaline); trypan blue (dyes slowly removed from the blood stream); phenolsulphonaphthalein; phenoltetrachlorophthalein; crystal S. scarlet and Buffalo fast crimson (dyes rapidly removed from the blood stream). (Refer to table 11.)

The measured amount of dye (20 mgm. per kilo body weight) is slowly injected and two 10 cc. samples of blood withdrawn at 4 and 40 minutes after injection and one 10 cc. sample 1, 2, 3, 4, 6, 8 and 12 hours after injection if the dye still persists in the plasma. After 12 hours, samples are taken on successive days at approximately the same hour until the amount of dye in the plasma is too small for satisfactory colorimetric determination. Dilution of the plasma is necessary for the first specimens which are all read against the usual standard prepared as described above.

In the following list of dye substances three main groups are made as follows:

Group I. Dyes behaving in the blood stream like brilliant vital red (blood volume group).

Group II. Dyes which show rapid disappearance from the blood plasma and are excreted in large measure by the kidneys. (Type of phenolsulphonaphthalein).

Group III. Dyes rapidly lost from the plasma yet not excreted by the kidneys. Certain dyes of this group show a substantial trace in the urine. (Intermediate group.)

#### LIST OF DYE SUBSTANCES

##### *Group I. Blood volume group*

<i>Designation</i>	<i>Chemical constitution</i>
Brilliant vital red.....	Orthotolidin + 1 mol. $\beta$ naphthylamine 3.6 disulphonic acid and 1 mol. $\beta$ naphthylamine 6 monosulphonic acid.
Vital new red (E and S).....	A tetrazo dye belonging to a group made by combining urea derivatives with amido naphthols, naphthols and naphthylamines.
Vital new orange (E and S).....	A tetrazo dye belonging to a group made by combining urea derivatives with amido naphthols, naphthols and naphthylamines.
No. 176.....	Tetrazo dye formed by combining para para di-amido diphenyl ureas with various naphthols, amido naphthols and naphthylamines.
Trypan red.....	Benzidine monosulphonic acid combined with 2 mol. $\beta$ naphthylamine 3.6 disulphonic acid.

Dianil granat B.....	Bensidine combined with 1 mol. 2 amido-8 naphthol 6 monosulphonic acid and 1 mol. $\beta$ naphthylamine 3.6 disulphonic acid.
No. 86.....	Bensidine combined with 1 mol. $\beta$ naphthylamine 3.6 disulphonic acid and 1 mol. $\beta$ naphthylamine 6 monosulphonic acid.
Columbia blue R.....	Bensidine combined with 1 mol. $\alpha$ naphthol 3.8 disulphonic acid and 1 mol. 1.8 amido naphthol 4 disulphonic acid.
No. 276.....	Bensidine combined with 1 mol. H acid and 1 mol. 2.8 ethyl amido naphthol 6 monosulphonic acid.
Direkt himmelbau gruenlich.....	Dianisidine combined with 2 mol. 1.8 amido naphthol 2.5 disulphonic acid.
Chicago blue 4B.....	Dianisidine combined with 1 mol. 1.8 amido naphthol 2.4 disulphonic acid and 1 mol. 1.8 amido naphthol 4 monosulphonic acid.
Chicago blue 6B.....	Dianisidine combined with 2 mol. 1.8 amido naphthol 2.4 disulphonic acid.
No. 2826 B.....	Dianisidine combined with 1.8 amido naphthol 2.4 disulphonic acid.
Hoechst No. 9.....	Ortho tolidin combined with 2 mol. $\beta$ naphthylamine 6 monosulphonic acid.
No. 204.....	Ortho tolidin combined with 1 mol. $\beta$ naphthylamine and 1 mol. $\beta$ naphthylamine 6 monosulphonic acid.
T-148.....	Ortho tolidin + 2 mol. $\alpha$ naphthylamine 4.8 disulphonic acid.
O-Tolidin + 1 NH <sub>2</sub> (4.8).....	Ortho tolidin + 2 mol. $\alpha$ naphthylamine 4.8 disulphonic acid.
Brilliant purpurine R.....	Ortho tolidin combined with 1 mol. $\alpha$ naphthylamine 4 monosulphonic acid and 1 mol. $\beta$ naphthylamine 3.6 disulphonic acid.
T + 2 mol. 1 naph. 3.6 disulf.....	Ortho tolidin + 2 mol. $\alpha$ naphthylamine 3.6 disulphonic acid.
T + 2 mol. $\beta$ naph. 5.7 disulf.....	Ortho tolidin + 2 mol. $\beta$ naphthylamine 5.7 disulphonic acid.
T-1824.....	Ortho tolidin combined with 2 mol. 1.8 amido 2.4 disulphonic acid.
No. 2826 A.....	Ortho tolidin combined with 2 mol. 1.8 amido 2.4 disulphonic acid.
2 mol. 1824 - 1 mol. O-tolidin.....	Ortho tolidin combined with 2 mol. 1.8 amido naphthol 2.4 disulphonic acid.
Dianil blue 2 R.....	Ortho tolidin combined with 1 mol. chromatrope acid and 1 mol. $\alpha$ naphthol 4 monosulphonic acid.
T + 1835 (alkaline).....	Ortho tolidin combined with 2 mol. 1.8 amido naphthol 3.5 disulphonic acid in alkaline solution.

T + 2 mol. 1846.....	Ortho tolidin combined with 2 mol. 1.8 amido naphthol 4.6 disulphonic acid.
Columbia blue G.....	Ortho tolidin combined with 1 mol. $\alpha$ naphthol 3.6 disulphonic acid and 1 mol. 1.8 amido naphthol 4 monosulphonic acid.
Wasserblau.....	Sulphonic acid of triphenyl para rosaniline.
Indazarun B. B.....	Dianisidine combined with 1 mol. 1.7 dioxy-2 naphtholic acid-4 sulphonic acid and 1 mol. $\beta$ naphthol 3.6 disulphonic acid.
No. 173.....	Tetrazo dye formed by combining para para diamido diphenyl ureas with various naphthols, amido naphthols and naphthylamines.

*Group II. Renal excretion group*

Phenolsulphonephthalein

Buffalo fast crimson.....	Mono azo dye formed by linkage of acetyl $\beta$ phenylendiamine combined with $\alpha$ naphthol 3.6 disulphonic acid.
Crystal s. scarlet.....	Mono azo dye formed by combining $\alpha$ naphthylamine with $\beta$ naphthol 6.8 disulphonic acid.
Tolan red.....	Mono azo dye formed by combining aniline with 1.8 amido naphthol 4.6 disulphonic acid.
No. 226.....	Benzidine disulphonic acid with 2 mol. 1.8 amido naphthol 4.6 disulphonic acid.
No. 227.....	Benzidine disulphonic acid with 2 mol. 2.8 amido naphthol 6 monosulphonic acid.
No. 228.....	Benzidine disulphonic acid with 2 mol. 2.5 naphthol 7 monosulphonic acid.
T-1835.....	Ortho tolidin combined with 2 mol. 1.8 amido 3.5 disulphonic acid (probably in acid solution).
No. 225.....	Benzidine meta disulphonic acid + 2 mol. H acid.
No. 105.....	Benzidine combined with 2 mol. chromatrope acid.
L. T. 297.....	Ortho tolidin disulphonic acid combined with 2 mol. amido naphthol 3.6 disulphonic acid.
T. disulfosaure + H acid.....	Ortho tolidin disulphonic acid combined with 2 mol. amido naphthol 3.6 disulphonic acid.
Alizarin green S.....	A mixture of tri and tetra oxy anthraquinone quinolines and their sulphonic acid derivatives.
No. 155.....	Para para diamido stilbene combined with 2 mol. H acid.
Indigo disulfosaure.....	Indigo disulphonic acid.



*Group III. Intermediate group*

New Bordeaux L.....	Benzidine combined with 2 mol. $\beta$ naphthol 8 monosulphonic acid.
Baumwoll rubin.....	Benzidine combined with 1 mol. $\alpha$ naphthylamine 4 monosulphonic acid and 1 mol. $\beta$ naphthol 8 monosulphonic acid.
Naphthamine black C. E.....	Benzidine combined with 1 mol. of 2.8 amido naphthol 6 monosulphonic acid and 1 mol. H acid.
Naphthamine black R. E.....	Benzidine combined with 1 mol. 2 amido 8 naphthol 6 monosulphonic acid + 1 mol. of the K acid.
Trisulfon violet.....	Benzidine combined with 1 mol. $\beta$ naphthol and 1 mol. $\alpha$ naphthol 3.6.8. trisulphonic acid.
No. 114.....	Benzidine combined with 1 mol. H acid and 1 mol. $\alpha$ naphthol 4 monosulphonic acid.
No. 316.....	Benzidine combined with 1 mol. H acid and 1 mol. 2.8 ethyl amido naphthol 6 monosulphonic acid.
No. 221.....	Dianisidine combined with 1 mol. Neville-Winther acid and 1 mol. 1.7 dioxy 2 naphthoe 4 sulphonic acid.
No 295.....	Dianisidine combined with 1 mol. $\alpha$ naphthol 4 monosulphonic acid and 1 mol. 1.7 dioxy 2 naphthoe 4 disulphonic acid.
No. 481 Mulheim blue II..	Ortho tolidin combined with 2 mol. chromotrope acid.
Lichtgrün S. F.....	Triphenyl methane dye. Chlor methylate of hexa methyl para rosaniline chloride.
Trypan blue.....	Benzidine dye: Ortho tolidin combined with 2 mol. 1.8 amido naphthol 3.6 disulphonic acid.
Congo blue (X pure) .....	Ortho tolidin combined with 1 mol. $\alpha$ naphthol 4 monosulphonic acid and 1 mol. H acid.
No. 222.....	Dianisidine combined with 1 mol. H acid and 1 mol. 1.7 dioxy 2 naphthoeic acid 4 sulphonic acid.
No 181.....	Tetrazo dye formed by combining para para diamido diphenyl ureas with various naphthols, amido naphthols and naphthylamines.
No. 230.....	Benzidine disulphonic acid combined with 2 mol. $\beta$ naphthylamine 7 monosulphonic acid.
No. 246.....	Tetrazo dye formed by combining para para diamido diphenyl ureas with various naphthols, amido naphthols and naphthylamines.

## EXPERIMENTAL OBSERVATIONS

It will be seen from table 1 that the different vital red dyes show considerable differences in the color intensities of the standard 1 per cent solution. Whether this may be due in part to inert salts present in the dye powder is not determined. It will be seen that the dyes may differ slightly in their physiological reaction in the plasma (table 3). For example, brilliant vital red II leaves the circulation with slightly greater speed than some of the other vital red dyes.

TABLE 1  
*Comparison of vital red dyes*

DESIGNATION	CHEMICAL CONSTITUTION	COLOR STRENGTH
		<i>per cent</i>
Brilliant vital red (new)	Ortho tolidin combined with one molecule $\beta$ naphthylamine 3.6 disulphonic acid and one molecule $\beta$ naphthylamine 6 monosulphonic acid	100
Brilliant vital red (old)	Ortho tolidin combined with one molecule $\beta$ naphthylamine 3.6 disulphonic acid and one molecule $\beta$ naphthylamine 6 monosulphonic acid	75
Brilliant vital red II	Ortho tolidin combined with one molecule $\beta$ naphthylamine 3.6 disulphonic acid and one molecule $\beta$ naphthylamine 6 monosulphonic acid	50
Original Rowntree dye	Diamino dixylyl methane combined with two molecules $\beta$ naphthol 3.6 disulphonic acid	60
Rowntree II no. 273	Dichlor benzidine combined with 2 molecules $\beta$ naphthylamine 3.6 disulphonic acid	100

Standardization of the six large dogs by means of these vital red dyes is detailed in table 2. It will be noted that these standard observations were made at intervals during the period in which these dogs were used to standardize the other dyes as indicated in other tables to follow. Table 2 may be used as a reference table to show the average blood volume figures as well as maximal and minimal readings for blood volume in individual dogs as determined by the vital red dye group. These same standardized dogs are used throughout the entire series of observations tabulated below. It is interesting to note the average figure for blood per kilo equals 93 and average cell hematocrit equals 50.4 per cent. It

may be stated again that these dogs were all strong, well-nourished, rather inactive adult males.

The speed with which these vital red dyes are removed from the circulating plasma can be estimated from the color readings given in table 3. During a period of 40 minutes on the average the color readings fall 13 per cent—that is, from an average reading of 90 color per cent to an average of 77 color per cent. There is good evidence that

TABLE 2  
*Blood volume determinations with vital red dyes*

DATE	DYE	DOG	WEIGHT	RED CELLS	PLASMA	COLOR (4 MIN.)	PLASMA VOL- UME	BLOOD VOL- UME	BLOOD PER KILOGRAM
			kgm.	per cent	per cent	per cent	cc.	cc.	
11/18	Brilliant vital red (new).....	16-178	27.04	43.2	55.1	89	1215	2206	82
11/26	Brilliant vital red (old).....	16-178	27.10	46.4	53.1	99	1095	2063	76
2/27	Brilliant vital red II.....	16-178	28.45	46.6	52.4	94	1210	2309	81
12/15	Original Rowntree dye.....	16-178	26.95	44.0	54.5	99	1088	1996	74
12/30	Brilliant vital red (new).....	18-60	21.90	48.6	50.4	83	1055	2093	91
11/22	Brilliant vital red (old).....	18-60	22.30	51.2	47.7	95	939	1968	88
11/29	Original Rowntree dye.....	18-60	21.80	52.7	46.3	82	1063	2295	105
2/25	Original Rowntree dye.....	18-60	21.90	47.5	51.5	76	1152	2236	102
11/21	Brilliant vital red (new).....	19-63	21.60	50.0	48.8	80	1080	2213	102
11/30	Brilliant vital red (old).....	19-63	21.90	53.6	45.4	87	1006	2215	101
12/28	Original Rowntree dye.....	19-63	23.00	50.2	48.6	80	1152	2370	102
11/23	Brilliant vital red (new).....	19-70	21.30	52.8	46.1	94	906	1965	92
4/29	Brilliant vital red II.....	19-70	26.80	45.8	53.7	99	1083	2017	75
5/6	Brilliant vital red (new).....	17-154	26.90	54.4	45.1	96	1123	2490	92
4/30	Brilliant vital red II.....	17-154	27.00	53.8	45.2	95	1137	2515	93
3/4	Brilliant vital red (new).....	19-71	24.10	53.4	46.1	96	1004	2193	91
1/22	Brilliant vital red II.....	19-71	23.50	54.8	45.2	86	1093	2418	103
2/11	Rowntree II no. 273.....	19-71	23.50	51.4	47.6	81	1160	2437	104
3/31	Rowntree II no. 273.....	19-71	25.50	51.7	47.2	88	1160	2457	96
Average.....			24.4	50.4	48.6	89			93

the dye is thoroughly mixed with the circulating blood at the end of 2 minutes as these readings are so constant when compared with the 4-minute samples which average 1 color per cent less. All the blood volume determinations are figured from the 4-minute reading which is arbitrarily taken as the optimum figure.

The data on color concentration in blood plasma at varying time intervals are presented in table 3 but the same data are given in different

TABLE 3  
Color determinations vital red dyes readings in per cent

DYE (DESIGNATION).....	BRILLIANT VITAL RED (NEW)						BRILLIANT VITAL RED (OLD)		BRILLIANT VITAL RED II				ORIGINAL ROWNTREE DYE			ROWNTREE DYE II	
	16-178	18-60	19-63	19-70	17-154	19-71	18-60	19-63	16-178	19-70	17-174	19-71	18-60	18-60	18-63	19-71	19-71
Dog number.....																	
Weight, pounds.....	59.5	48	47.5	47.0	59	53	49.13	48.26	62.5	59	59.5	51.5	48.0	48.13	50.75	51.75	56.0
Time after injection readings in per cent <div> <div>2 min.</div> <div>4 min.</div> <div>6 min.</div> <div>10 min.</div> <div>15 min.</div> <div>25 min.</div> <div>40 min.</div> </div>	90	85	88	95	97	97	99	90	94	100	95	88	85	77	80	81	90
	89	83	86	94	96	96	95	87	94	99	95	86	82	76	80	81	88
	88	83	84	94	95	96	88	86	93	97	93	85	82	75	79	81	87
	84	83	80	94	93	95	86	83	91	95	88	83	81	73	77	80	85
	83	82	77	90	90	93	85	80	88	92	85	81	80	71	75	78	83
	81	80	73	86	88	91	83	78	81	87	81	74	77	70	71	76	80
	79	79	70	79	87	89	79	76	76	83	77	70	73	68	68	75	77
Total loss in color per cent— 40 minutes.....	11	6	18	16	10	8	20	14	18	17	18	18	12	9	12	6	13

form in table 4 to show the loss in color per cent reading during the time intervals as tabulated. There is practically always a slight fall in color readings in the interval between the 2- and 4-minute samples (table 4—first column, 2 to 4)—average 1.4 color per cent. The same fall is noted in the interval between the 4- and 6-minute samples—average 1.2 color per cent. The loss of dye from the plasma is less

TABLE 4

*Decrease in color concentration of plasma; minutes after injection*

DYE (DESIGNATION)	DOG	2 TO 4 MIN-UTES	4 TO 6 MIN-UTES	6 TO 10 MIN-UTES	10 TO 15 MIN-UTES	15 TO 25 MIN-UTES	25 TO 40 MIN-UTES	TOTAL 40 MIN-UTES COLOR PER CENT
Brilliant vital red (new) . . .	18-60	2	0	0	1	2	1	6
	19-63	2	2	4	3	4	3	18
	16-178	1	1	4	1	2	2	11
	19-70	1	0	0	4	4	7	16
	17-154	1	1	2	3	2	1	10
	19-71	1	0	1	2	2	2	8
Brilliant vital red (old) . . . . .	18-60	4	7	2	1	2	4	20
	19-63	3	1	3	3	2	2	14
Brilliant vital red II . . . . .	16-178	0	1	2	3	7	5	18
	19-70	1	2	2	3	5	4	17
	17-154	0	2	5	3	4	4	18
	19-71	2	1	2	2	7	4	18
Original Rowntree . . . . .	18-60	3	0	1	1	3	4	12
	18-60	1	1	2	2	1	2	9
	19-63	0	1	2	2	4	3	12
Rowntree II no. 273 . . . . .	19-71	0	0	1	2	2	1	6
	19-71	2	1	2	2	3	3	13
Average . . . . .		1.4	1.2	2.1	2.2	3.3	3.1	13.3

rapid as we approach the 40-minute period—for example, a loss of 3.1 color per cent during 15 minutes preceding the 40-minute reading. During the first 10 minutes after the dye injection there is an average loss in color per cent reading of 1 per cent per 2-minute intervals. It will be noted that brilliant vital red (old) and (II) leave the blood stream a little more rapidly on the average than do the other dyes in this group. A part of this difference may be apparent and not real.

TABLE 5  
Color determinations for dyes of group I A: readings in per cent

DYE .....	T-1824			TWO MOLE- CULES 1824 ONE MOLE- CULE O-TOLUIDIN			No 2826A			T-1835 (ALKALINE)			CHICAGO BLUE 6B			DIANIL BLUE 2R			No. 2826B			ROSCHE 9			No. 173		
	18-60	19-63	17-154	19-63	19-71	17-154	17-154	16-178	17-154	17-154	18-60	19-63	18-60	19-71	19-70	19-71	19-70	19-71	17-154	17-178	19-70	19-71	18-60	19-71	18-60	19-71	
Dog number.....	48	49.25	61-0	49-25	55.0	60.5	60.5	60.5	59.63	47.38	52.63	47.0	49.5	50.25	51.0	47.5	58.75	58.75	63.13	45.63	56.5	51.5	51.5	51.5	51.5		
Weight in pounds.....	78	83	98	90	97	104	106	106	90	76	76	76	97	92	84	97	110	110	87	74	98	85	85	85			
Time after injection reading in per cent.....	2 min.	78	83	98	90	96	102	104	89	75	75	75	97	91	83	96	110	110	87	73	97	84	84	84			
	4 min.	78	82	96	89	95	100	102	89	74	74	74	96	90	82	95	108	108	87	73	96	82	82	82			
	6 min.	77	80	95	87	93	99	100	87	74	73	74	95	88	81	88	106	106	85	71	94	81	81	81			
	10 min.	77	79	95	83	91	97	99	86	72	71	72	95	86	80	88	106	106	82	67	90	80	80	80			
	15 min.	77	75	93	78	88	97	99	84	70	68	71	94	84	75	84	106	106	75	58	88	77	77	77			
25 min.	70	74	91	74	85	95	98	98	83	69	64	68	93	81	70	83	104	104	71	54	83	72	72	72			
40 min.																											
Total loss in color per cent in 40 minutes.....	8	9	7	16	12	9	8		7	7	12	8	4	11	14	14	6	6	16	20	15	13	13	13			

These two dyes are paler than the others and the color readings therefore a little less accurate. The differences, however, are too small to have much significance.

In group I-A are included seven dyes which compare favorably with the vital red series and may be substituted if occasion arises. There are five blue dyes in this group and the blue color in itself has certain advantages over the red dye colors. Many workers can estimate blue colors more accurately, more rapidly and with less fatigue than is the case with red colors. Slight hemolysis can be recognized more easily when the blue dyes are used. Even a trace of hemolysis will rarely confuse one who is thoroughly familiar with the blood volume work no matter what dye is being used.

One blue dye is particularly well adapted to the routine blood volume work and is preferred by us when compared with the vital red group. The difference, however, is not great but for many workers the blue dye has advantages. This azo dye is tested in table 5 in seven different experiments. Three different samples of the same dye are used (T-1824 and 2-mol. 1824, 1 mol O-tolidin and No. 2826 A) and tabulated in the first three groups of table 5. It will be noted that this blue dye is slow to leave the circulating blood plasma, if anything slightly more so than the vital red series.

The second blue dye (T-1835 alkaline) is also slow to leave the blood stream during the observed period of 40 minutes but the three color readings are somewhat lower than the usual average. We are not prepared to explain this point but many more observations must be made before any such unusual reaction can be accepted.

AVERAGE COLOR CONCENTRATION	VITAL RED	T-1824	T-1835 (ALKALINE)
Two-minute samples.....	90	94	81
Four-minute samples . . . . .	88	93	80
Forty minute samples....	78	84	72

The above tabulation shows that the vital red figures compare closely with the blue azo dye T-1824. The readings of T-1835 (alkaline) are low but these figures represent the average of too few observations while the vital red figures represent a great many experiments.

It will be seen that the curve of dye removal from the blood stream is the same for this group of dyes as for the vital red group. If anything these dyes (group I-A) are even more slowly removed from the blood stream, particularly the blue dyes.

The two red dyes (Hoechst 9 and No. 173) of group 1-A behave very much like the vital red group. Too few observations have been made to determine this point beyond question.

TABLE 6  
*Decrease in color concentration of plasma for dyes of group I-A.  
Minutes after injection*

DYE	DOG	2 TO 4 MIN-UTES	4 TO 6 MIN-UTES	6 TO 10 MIN-UTES	10 TO 15 MIN-UTES	15 TO 25 MIN-UTES	25 TO 40 MIN-UTES	TOTAL 40 MIN-UTES COLOR PER CENT
T-1824.....	18-60	0	0	1	0		7	8
	19-63	0	1	2	1	4	1	9
	17-154	0	2	1	0	2	2	7
2 mol. 1824, 1 mol. O-tolidin	19-63	0	1	2	4	5	4	16
	19-71	1	1	2	2	3	3	12
No. 2826 A.....	17-154	2	2	1	2	2	2	9
	16-178	2	2	2	1	0	1	8
T-1835 (alkaline).....	17-154	1	0	2	1	2	1	7
	18-60	1	1	0	2	2	1	7
	19-63	1	1	1	2	3	4	12
Chicago blue 6B.....	18-60	1	0	1	2	1	3	8
	19-71	0	1	1	0	1	1	4
Dianil blue 2R.....	19-71	1	1	1	1	5	5	14
	19-70	1	1	2	2	2	3	11
No. 2826 B.....	19-70	1	1	7	0	4	1	14
	17-154	0	2	2	0	0	2	6
Hoechst 9.....	16-178	0	0	2	3	7	4	16
	19-70	1	0	2	4	9	4	20
No. 173.....	19-71	1	1	2	4	2	5	15
	18-60	1	2	1	1	3	5	13

The corresponding blood volumes on the standardized dogs are figured in table 7 and are found to be in harmony with observations made with vital red dyes in table 2. The first experiment in table 7 gives a figure of 70 cc. per kilo for the blood volume which is open to



criticism. This figure is very low as compared with many others on this same dog and is much below the average. It is more than probable that some technical error crept into this experiment—possibly some dye contaminating a container, syringe or hypodermic needle.

Group I-B contains many dyes which are fairly satisfactory in many respects when used for blood volume determinations. These dyes do not leave the blood stream rapidly and in only one or two instances

TABLE 7  
*Blood volume determinations group I-A*

DATE	DYE	DOG	WRIGHT	RED CELLS	PLASMA	COLOR PER CENT 4 MINUTES	PLASMA VOL- UME	BLOOD VOL- UME	BLOOD PER KILOGRAM	DECREASE IN COLOR CONCENTR- ATION 40 MINUTES
			mgm.	per cent	per cent		cc.	cc.		
1/15	No. 2826A	16-178	27.5	44.2	54.8	104	1058	1930	70	9
1/2	Hoechst No. 9	16-178	28.2	48.1	50.9	87	1298	2550	90	16
12/9	T + 1824	18-60	21.8	50.2	48.8	78	1120	2295	105	8
2/3	T + 1835 (alkaline)	18-60	21.5	49.5	49.5	75	1149	2321	107	7
1/7	Chicago 6 B	18-60	21.3	49.5	50.0	75	1138	2276	106	8
6/30	No. 173	18-60	23.4	48.4	50.1	84	1116	2227	95	13
12/7	T + 1824	19-63	22.4	52.6	45.8	83	1079	2356	104	9
1/22	2 mol. 1824 + 1 mol. O-tolidin	19-63	22.4	54.0	45.0	90	995	2211	98	16
3/1	T + 1835 (alkaline)	19-63	23.7	51.0	48.0	75	1266	2637	111	12
2/5	Dianil blue 2 R	19-70	23.7	46.1	52.9	91	1044	1973	83	11
1/11	No. 2826 B	19-70	21.6	43.7	55.3	96	900	1627	96	14
1/4	Hoechst No. 9	19-70	20.2	45.8	53.2	73	1109	2064	102	20
3/3	T + 1824	17-154	27.7	54.5	44.5	98	1132	2544	92	7
1/10	No. 2826 A	17-154	27.5	54.6	44.4	102	1078	2428	88	9
2/1	T + 1835 (alkaline)	17-154	27.1	60.5	39.5	89	1195	3026	111	7
1/16	No. 2826 B	17-154	26.7	62.5	36.5	110	970	2657	99	6
5/2	2 mol. 1824 + 1 mol. O-tolidin	19-71	25.0	53.8	45.7	96	1042	2280	91	12
1/14	Chicago blue 6 B	19-71	22.5	55.6	43.4	97	928	2138	95	4
2/4	Dianil blue 2 R	19-71	23.2	55.2	44.3	83	1118	2523	109	14
6/10	No. 173	19-71	25.7	57.2	41.8	97	1059	2533	99	15

are moderately toxic. As a group the colors are pale and correspondingly difficult to read. A pale color cannot be as accurately determined and work with such dyes is more time consuming. It should be stated, however, that trypan red, indazarun B. B. and no. 204 when injected in the strength of 4 mgm. per kilo do give satisfactory colors for routine work. Used in this strength these three dyes compare favorably with the vital red group.

TABLE 8  
Blood volume determinations, group I-B

DATE	DYE	DOG	WEIGHT	RED CELLS	PLASMA	COLOR PER CENT 4 MINUTES	PLASMA VOL-UME	BLOOD VOL-UME	BLOOD PER KILOGRAM	DECREASE IN COLOR CONCENTRATION 2-40 MINUTES
			kgm.	per cent	per cent		cc.	cc.		
10/14	Vital new red	16-178	28.0	45.5	53.4	104	1077	2016	72	21
1/8	Chicago blue 4B	16-178	28.0	48.8	50.2	104	1077	2145	76	15
1/23	No. 204	16-178	28.5	45.5	53.5	98	1163	2173	76	22
1/30	Indazarun B.B.	16-178	28.2	50.9	49.0	97	1163	2373	84	11
1/17	Vital new red	18-60	21.2	49.5	50.0	87	977	1940	91	8
5/15	No. 176	18-60	24.8	46.3	52.7	91	1090	2068	82	10
4/26	No. 86	18-60	24.5	45.3	53.7	82	1195	2225	90	10
3/5	No. 204	18-60	22.1	49.0	50.5	79	1119	2215	100	18
3/22	T - 148	18-60	23.0	47.1	51.9	93	989	1905	83	8
12/16	T + 2 mol. $\beta$ naphth. 5.7 disulph.	18-60	21.5	50.2	48.7	85	1011	2076	96	13
2/21	Trypan red	19-63	23.0	50.3	48.7	73	1263	2593	112	12
12/14	Direkt himmel blau gruenlich	19-63	23.0	50.7	47.9	79	1167	2436	105	13
1/13	Chicago blue 4 B	19-63	22.4	55.8	43.2	87	1030	2384	106	8
12/21	T + 2 mol. 1846	19-63	23.8	49.5	49.5	72	1325	2676	113	8
1/31	Indazarun B.B.	19-63	21.7	54.1	44.9	77	1114	2481	114	14
6/27	No. 176	19-70	25.7	57.0	41.9	114	901	2150	83	14
2/19	Trypan red	19-70	23.6	42.2	56.8	93	1017	1790	75	8
12/24	T + 2 mol. 1 naph. 3.6 disulf.	19-70	21.3	35.3	63.7	83	1265	1985	93	23
12/17	T + 2 mol. 1 naph. 3.6 disulf.	19 70	21.7	44.8	54.2	92	945	1743	80	9
	No. 86	17-154	27.5	53.2	45.8	95	1157	2526	92	7
6/28	No. 276	17-154	27.7	58.6	40.4	99	1122	2777	100	21
12/13	Direkt himmelblau gruenlich	17-154	27.7	55.5	43.4	97	1144	2635	94	5
12/20	2 mol. $\beta$ naph. 5.7 di-sulfosaure	17-154	27.7	55.7	43.7	102	1088	2489	89	14
4/16	Wasserblau	17-154	27.0	53.2	46.2	85	1165	2502	92	8
5/16	No. 276	19-71	25.2	52.2	47.2	90	1122	2377	94	18
3/25	T - 148	19-71	25.0	52.8	46.7	92	1087	2327	93	7
12/18	T + 2 mol. 1846	19-71	23.6	51.1	48.3	79	1197	2478	104	5
4/15	Wasserblau	19-71	25.3	53.8	45.7	100	1014	2219	87	13

Wasserblau is decolorized by the blood plasma but the color returns on the addition of 2 drops of concentrated hydrochloric acid to blood serum tubes and standard. Brilliant purpurine in the strength of 4

mgm. per kilo will cause hemolysis in the circulating blood, and some clinical evidence of intoxication.

Vital new red, vital new orange, direkt himmelblau gruenlich and T-148 persist in the blood stream for 7 to 10 days or longer. This is an objection to the routine use of these four dyes and may introduce difficulties when a series of observations is to be made upon the same animal.

As regards the dyes in group I-B, too few observations have been made to settle all points of interest which concern blood volume work. Some single observations are not even included in table 8 as they show nothing unusual and the dyes gave no promise of being useful in this work. This statement applies to columbia blue R, O-tolidin+1 NH<sub>2</sub> (4.8), dianil granat B, brilliant purpurine R, columbia blue G, and some others listed but not specifically mentioned.

TABLE 9  
*Summary. Dyes of group I*

	VITAL RED DYES 19 EXPERIMENTS			OTHER DYES OF GROUP I-A 20 EXPERIMENTS			DYES IN GROUP I-B 28 EXPERIMENTS		
	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum
Kilogram weight.....	24.4	21.3	28.45	24.1	21.3	28.25	24.7	21.2	28.5
Red cells per cent.....	50.4	43.2	54.8	51.8	43.7	62.5	50.1	35.3	58.6
Color per cent (4-minute sample)	89	76	99	89	73	110	90.3	72	114
Blood per kilogram body weight	93	74	105	92	70	111	92	72	114
Decrease in color per cent 2-40 minutes	13	6	20	11	6	20	12	5	23

### *Dyes of group II*

This group includes the dyes which are eliminated more or less completely by the kidneys. Phenolsulphonephthalein is a dye familiar to everyone and we record in table 10 familiar data concerning renal excretion of this dye. The amounts injected are somewhat larger than the usual clinical dose but the per cent excretion is normal for dogs. The rapid disappearance from the blood stream is well shown. Much of the phthalein has left the blood by the end of 2 minutes and almost all of it by the end of the next 4 minutes. Phenoltetrachlorophthalein (1) which is known to be excreted in the bile is taken out of

the blood under similar conditions with even greater speed. There is practically complete removal of this dye from the blood serum by the end of 2 minutes following its injection. Perhaps the relatively greater size of the liver and consequent larger minute circulation volume as compared with the kidneys may account for this difference.

Several of the dyes listed in table 11 (group II) are rapidly removed from the blood stream but none with the speed just recorded for phenol-sulphonephthalein. Buffalo fast crimson, crystal S. scarlet, tolan red, T-1835 and indigo disulfosaure are dyes which show minimal color readings in the plasma 6 minutes after the dye injection. Probably the dye is almost completely removed within a period of 15 minutes.

TABLE 10

*Decrease in color concentration of plasma and excretion by the kidneys—  
Phenolsulphonephthalein\**

DATE	DOG	WEIGHT	COLOR PER CENT 2 MINUTE SAMPLE	LOWEST COLOR PER CENT RECORDED	TIME INTERVAL OF LOWEST COLOR PER CENT	TOTAL DYE RECOV- ERED IN URINE	TIME RECOVERY OF DYE IN URINE
		<i>kilograms</i>			<i>minutes</i>	<i>per cent</i>	<i>hours</i>
5/7	16-178	24.65	26	18	4	62.8	4
5/8	18-60	24.5	38	27	4	72.7	4
5/7	19-63	22.8	28	20	4	60.5	6
12/10	19-70	21.3	41	28	4	60.0	2
12/6	17-154	27.0	50	38	4	70.3	3
5/8	19-71	25.5	35	23	4	64.9	4
Average .....		24.3	36	26		65.2	

\* 1.3 mgm. per kilo body weight.

Buffalo fast crimson seems to be more like phenolsulphonephthalein in that over 50 per cent of the dye is excreted in the urine. It seems that this dye should be more carefully studied as to its excretion by the kidney in health and disease. The other dyes show wide variations in the amount recovered from the urine after intravenous injection. It is possible that conditions present in the kidney, bladder or urine may modify the yield to a considerable extent. A more careful study of this group of dyes should be undertaken and may yield information of value concerning the secretory activity of normal and abnormal cells as concerns these particular dyes. It will be of some interest to determine whether the same or different limitations apply to all these dyes as is true for phenolsulphonephthalein.

The other dyes in group II leave the blood stream less rapidly and in most instances a readable amount of dye remains at the end of 40

TABLE 11  
*Dyes of group II*

DATE	DYE	DOG	WEIGHT	COLOR PER CENT 2 MIN- UTES	LOWEST COLOR PER CENT RE- CORDED	TIME INTERVAL OF LOWEST COLOR PER CENT	TOTAL DYE RE- COVERED IN URINE	URINE COLLEC- TION
			kgm.			minutes	per cent	hours
12/12	T + 1835 (B 1841)	16-178	27.0	48	33	6	21.1	4
12/14	L. T. 297	16-178	27.75	78	54	40	14.4	3
11/1	Alizarin green	16-178	26.76	61	39	45	24.8	3
3/6	No. 227	16-178	29.0	65	36	40	8.1	6
5/23	Buffalo fast crimson	18-60	25.0	61	35	15	53.0	3
11/13	Crystal s. scarlet	18-60	22.3	63	44	6	20.6	2
4/12	Indigo disulfosäure	18-60	23.8	42	20	6	53.4	3
12/23	L. T. 297	18-60	22.2	77	54	40	15.2	4
4/19	No. 225	18-60	24.5	65	36	40	17.2	4
1/2	No. 228	18-60	21.3	73	48	40	8.8	4
6/7	Crystal s. scarlet	19-63	23.6	66	30	15	5.3	1
4/4	Indigo disulfosäure	19-63	23.5	55	35	6	22.3	4
1/6	No. 105	19-63	23.2	69	44	40	4.0	2
4/25	No. 225	19-63	24.0	55	23	40	14.3	4
3/4	No. 227	19-63	21.9	65	36	40	11.7	6
12/2	T - disulfosäure + H. acid	19-70	20.3	74	47	40	6.6	4
1/29	No. 226	19-70	21.9	72	37	40	20.7	6
11/27	T - 1835	17-154	27.1	50	37	6	15.4	4
1/3	No. 105	17-154	27.2	86	60	40	18.2	2
1/25	No. 228	17-154	26.9	75	55	40	6.2	3
12/3	Buffalo fast crimson	19-71	22.9	58	28	15	51.1	3
1/7	Tolan red 4 B	19-71	23.3	59	31	10	29.2	4
12/31	Tolan red 4 B	19-71	23.3	52	30	10	29.5	4
3/21	T + 2 mol. 1.8 amido naph. 3.5 disulfosäure	19-71	25.0	53	28	6	7.5	4
12/11	T + 1835 (B. 1841)	19-71	23.3	50	35	4	13.7	4
11/25	T + 1835	19-71	24.3	44	32	6	7.2	4
11/15	Alizarin green	19-71	23.4				16.0	1
1/28	No. 226	19-71	23.3	73	41	40	19.1	5

minutes. One is not surprised therefore to note that the dye elimination in the urine is scanty and rarely exceeds 15 to 20 per cent.

*Group III-A*

This group contains a number of dyes which exhibit peculiar reactions in the blood stream when compared with the dyes in group I. At present we have insufficient experimental data to give the correct explanation so that discussion of various possibilities will be brief.

Two dyes (new Bordeaux L and lichtgrün S. F.) leave the blood stream with considerable rapidity yet do not appear in the urine (table

TABLE 12  
*Dyes of group III-A*

DATE	DYE	DOG	WEIGHT	COLOR CONCENTRATION 2 MINUTES	LOWEST COLOR PER CENT RECORDED	TIME INTERVAL FOR LOWEST COLOR PER CENT
			kgm.	per cent		minutes
3/26	New Bordeaux L	16-178	27.0	62	35	10
3/20	Baumwoll rubin	16-178	27.0	86	48	40
3/13	Naphthamine black R. E.	16-178	28.3	77	41	40
3/19	New Bordeaux L	18-60	22.3	70	37	10
2/17	Naphthamine black R. E.	18-60	21.95	55	44	40
4/5	No. 413 trisulfon violet	18-60	24.5	50	40	40
4/11	Lichtgrün S. F.	19-63	24.0	75	43	10
4/18	No. 114	19-63	24.0	94	66	40
6/24	No. 316	19-63	24.1	68	34	40
6/30	No. 295	19-63	24.5	75	52	40
4/3	Mulheim blue	19-70	26.5	99	69	40
4/10	No. 413 trisulfon violet	19-70	26.7	55	33	40
4/17	No. 114	19-70	27.0	100	73	40
3/24	Baumwoll rubin	17-154	27.0	87	55	40
4/9	Mulheim blue	17-154	27.6	110	85	40
2/15	No. 221	17-154	27.5	99	70	40
3/10	No. 316	17-154	27.0	72	38	40
6/9	No. 295	17-154	27.5	95	71	40
4/8	Lichtgrün S. F.	19-71	25.25	79	39	10
3/	No. 221	19-71	24.0	94	55	25
2/17	Naphthamine black R. E.	19-71	24.0	82	54	40

12). We have not been able to test for elimination in the bile so this cannot be excluded. Lichtgrün S. F. was decolorized in the blood plasma. Two drops of acetic acid brought back the color but hydrochloric acid did not develop the color (compare wasserblau). New Bordeaux L. was tested *in vitro* against normal serum which did not influence the dye even after three days' incubation at 38°C. It is possible, of course, that the endothelial cells of the living body are concerned in this reaction.

Other dyes in table 12 show occasionally a figure for the 40-minute period indicating a slow removal but a low initial reading. This, however, is not constant and the low initial reading is perhaps an accident due to unknown factors. There is a possibility of dye removal by the process of coagulation—that is, the dye may be carried out of solution by the clot. Unfortunately this point has not been controlled in this group as has been done in the vital red group. When these experiments were begun this possibility was not considered and oxalate plasma was not used in place of coagulated serum. In view of the irregular figures noted in table 12 for the same dye we must consider this possibility until it is disproved.

TABLE 13  
Decrease in color concentration of plasma. Group III-B.  
Minutes after injection

DATE	DYE	DOG	2 TO 4 MIN- UTES	4 TO 6 MIN- UTES	6 TO 10 MIN- UTES	10 TO 15 MIN- UTES	15 TO 25 MIN- UTES	25 TO 40 MIN- UTES	TOTAL 40 MIN- UTES COLOR PER CENT
2/6	Congo blue B	16-178	2	0	2	3	13	5	25
2/20	No. 230	16-178	1	3	6	3	9	6	28
3/11	No. 230	18-60	4	3	4	3	4	6	24
6/25	No. 181	18-60	2	4	3	5	5	13	32
5/3	Trypan blue	19-63	3	3	6	3	3	6	24
2/7	Congo blue B	19-63	1	3	2	2	6	5	19
5/14	No. 246	19-63	4	3	7	5	5	5	29
5/12	No. 181	19-70	0	8	3	4	20	6	41
2/26	Trypan blue	19-71	0	1	2	3	4	12	22
6/24	No. 246	19-71	1	3	5	8	8	5	30

The dyes given in table 13 all give a rather low initial reading as well as a pretty rapid removal during the 40-minute period. It is possible at least that the endothelial cells are concerned in a part of this reaction. It should be noted that the urine usually shows a definite trace of dye but not enough for a colorimetric reading. Three dyes not tabulated have been tested (no. 222, Hoechst no. 229 and no. 258). All these dyes give colors in the blood serum which are too pale to be accurately estimated.

When large amounts of dye are injected into the blood stream (10 to 20 times the routine amount) the reaction is slightly different but the dyes of group I give constant figures (table 14). The vital red dyes are removed from the blood with somewhat more speed than the blue

dyes (T-1824 and T-1835, alkaline). Traces of the dye remain in the serum for days after the reading cannot be accurately determined; for example, after the injection of brilliant vital red II (table 14) the serum showed a distinct pink color on the 17th day after injection. Also a trace of this dye was excreted in the urine but the amount was too small for colorimetric reading. Following the large dose of brilliant vital red (new) the skin, mucous membranes and eye-lids became pink,

TABLE 14  
Decrease in color concentration with large dosage of certain dyes.  
All readings in per cent

TIME AFTER INJECTION	DOG				
	19-28 (20.13 pounds)	19-136 (14.6 pounds)	19-63 (52.5 pounds)	19-117 (42.5 pounds)	19-70 (53 pounds)
	Brilliant vital red II 20 mgm. per kilo	Brilliant vital red (new) 30 mgm. per kilo	T-1824 20 mgm. per kilo	T-1835 (alka- line) 20 mgm. per kilo	Trypan blue 40 mgm. per kilo
4 min.	71	70	72	71	
40 min.	63	61	67	67	
60 min.	61	58	65	62	
2 hrs.	55	50	60	55	49
4 hrs.	41	38	50	53	33
6 hrs.	38		45	49	24
8 hrs.	36	26.6	40	44	20
12 hrs.	30	19.3	29		15
2nd day	17.3	10.0	22	30.3	10
3rd day	11.0	4.4	12	15.0	7.6
4th day	5.3	2.1	10.2	12.0	5.6
5th day	2.0	1.4	8.2	9.0	4.6
6th day	1.2		5.0	6.3	3.2
7th day			4.0		
8th day			2.8		
9th day			1.6	3.8	
10th day					1.2

which color lasted only a few minutes. Months after injections of these vital red dyes we find traces of the dye in the mesenteric and other lymph glands which are stained a delicate pink.

The curve of dye elimination is incomplete for trypan blue because the large amount used (40 mgm. per kilo) was responsible for considerable hemolysis which obscured the readings for the first two hours. It is interesting to note the long period (10 days +) during which the dye is present in the blood serum.



Several dyes of group II were tested in the same way by the use of ten times the routine dose. In these experiments the rapid elimination of the dye from the blood is very striking.

Phenolsulphonephthalein (20 mgm. per kilo) shows a 4-minute blood serum reading 19 per cent and a fall to 4 per cent at the end of 40 minutes. Only 54 per cent of the dye was recovered in the urine during 24 hours and most of this during the first hour (dog 18-60). This same animal with a routine injection gave 72 per cent elimination in the urine.

Phenoltetrachlorophthalein (20 mgm. per kilo) shows a 4-minute blood serum reading of 7 per cent and a fall to 1.4 per cent at the end of 40 minutes. With smaller amounts of dye there is scarcely a trace of dye in the serum at the end of 4 minutes. With the large dose there was noted a mere trace of dye in the urine.

Buffalo fast crimson (20 mgm. per kilo) gives a higher color per cent for the 4-minute blood serum sample (35 per cent) but complete removal at the end of 40 minutes. After the large dose the urine contained 27 per cent of the dye but after 2 mgm. per kilo dye injection we note a dye elimination in the urine of 51 to 53 per cent.

Crystal S. scarlet (20 mgm. per kilo) gives a higher blood serum 4-minute reading (39 per cent) but also complete removal in 40 minutes. The urine contained only 7 per cent of the dye which was excreted almost completely during the first hour.

#### DISCUSSION

A summary of all dyes of group I appears in table 9. It is readily seen that there is a remarkable average for this group of standardized dogs tested by various groups of dyes. All groups give the same average figure of blood volume per kilo body weight as 92 to 93 cc. The speed of disappearance of dye from the blood stream is constant in the average for 40 minutes and reads 11 to 13 color per cent. This is really a striking uniformity in results and for this reason these figures cannot be put aside by any quibble about the action of colloids in the blood stream, adsorption phenomena, etc.

The outstanding fact remains as follows: Any one of a large series of dyes may be injected into the blood stream and within 2 minutes it is diluted to a certain color which remains almost unchanged for 4 to 6 minutes or longer. This indicates a circulating fluid volume of a certain bulk whatever the limiting structures of this area may be. We

cannot admit that a certain portion of the dye may escape from the blood capillaries or be phagocytocized during the first 2 minutes following the injection unless we admit the same or similar possibility for the second 2-minute interval. There is no such disappearance during this second period of 2 minutes, so no person without definite proof has the right to assume or postulate any such reaction during the first 2 minutes. If these facts established by these dye injections do not harmonize with our preconceived notions concerning blood volumes and fluids circulating in the living body, then it is time to modify these concepts.

#### SUMMARY

Blood volume measurement is not the property of a single dye substance or group of such substances but may be accomplished by a great number of dyes.

It is essential that the dye is non-toxic, and not stored in the tissues, that the colors are such as to permit accurate determination and that the dye is removed quite slowly from the blood stream. Given these characteristics a dye is suitable for blood volume work.

It is of some significance that all suitable dyes give a remarkably constant figure on the average for blood volume determinations (table 9). This may indicate that the fluid measured in the blood stream is relatively constant, whatever our mental reservations may be concerning the actual extent of this fluid medium or its limiting structures.

A blue azo dye (T-1824) in our hands is slightly superior to the vital red groups especially as regards ease and accuracy of colorimetric readings.

Several other dyes are studied and found to be satisfactory for routine blood volume work (table 5).

A great number of dyes are included in this study and are not suitable for blood volume work. Some are rapidly removed from the blood by way of the kidney or liver. The significance of these dye reactions cannot be discussed at this time.

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## BLOOD VOLUME STUDIES

### IV. BLOOD VOLUME AS DETERMINED BY THE CHANGE IN REFRACTIVITY OF THE SERUM NON-PROTEIN FRACTION AFTER INJECTION OF CERTAIN COLLOIDS INTO THE CIRCULATION

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This investigation forms a part of the general program of this laboratory which includes a study of the complex problem of blood volume determinations. This problem is being approached from all angles and other publications dealing with the subject will appear in the near future. It occurred to us that certain colloids having a high refractive index might be introduced into the blood stream with a corresponding change in the refractive index of the serum. Acacia and gelatin are used and are found to appear quantitatively in the non-protein fraction of the serum. The change in refractive index of the non-protein fraction is sufficient to permit accurate determination of the degree of dilution affected by the mixture of the colloid solution with the circulating blood. This permits us to estimate with considerable accuracy the circulating blood volume by which these colloids are diluted and in which they circulate through the body. It is significant that blood volume as determined by this method compares very closely to the blood volume as determined by the vital red dyes. This indicates that the fluid areas included in the vascular system as measured by these various substances are of similar extent.

It is not our intention to review the many papers dealing with the different phases of blood volume work. We shall refer to the work of Keith, Rowntree and Geraghty (1), who first used the "vital red" method. We shall also give frequent references and figures which cover some of the work done in this laboratory by the use of "brilliant vital red."

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The recent work of Meek and Gasser (2) is of particular interest to us. These workers introduce intravenously large amounts of acacia, then remove some blood and make the proper analysis for the contained acacia. It is necessary to hydrolyze the acacia to liberate the pentosan which can then be determined quantitatively as a phloroglucid. One of us (Davis) carried out a number of experiments using this method but in our hands this method presented certain difficulties—it is very time-consuming and the figures are not at once available. The final gravimetric determinations are carried out on such small amounts of material that slight discrepancies in weighing cause large percentage errors. We feel that the method is unique and in theory valid.

The value of the refractometer in biological analysis has been especially emphasized by Reiss (3). This work has been somewhat elaborated in this country by Robertson (4). Although various workers have solved and suggested the attack of many problems in blood analysis, we have seen no reference to the application of refractometry to the determination of colloidal additions to the blood stream in blood volume work.

A substance to be suitable for injection in our work must possess certain qualities: *a*, It should go into solution in rather high concentration. *b*, It must be relatively non-toxic and non-antigenic. *c*, It must remain unchanged in the circulation at least until thoroughly mixed. *d*, It should have a high refractive index. *e*, It should be recoverable quantitatively in the non-protein fraction of the serum.

Among the substances considered, gelatin and gum acacia were finally chosen as best suited to our work. Proteins other than gelatin were considered, but were rejected for various reasons which are obvious. Starch and inulin disappear from the blood stream too quickly. Agar agar gels too readily, is hard to get into a concentrated solution and may produce grave intoxication as demonstrated on guinea pigs by Novy and De Kruif (5). Gum tragacanth will not form a concentrated solution, and is quite expensive. Possibly other substances colloidal in nature may be suggested by other investigators if this general method finds favor.

Gelatin and acacia are both relatively cheap and easily obtained; they may be made into 20 per cent to 25 per cent solutions with little difficulty; they are non-toxic and non-antigenic, as shown by Starin (6) and others for gelatin, and as shown by Gasser and Meek (2) and by De Kruif (7) for acacia; both remain in the serum non-protein fraction; both disappear from the blood stream quite slowly as Buglia (8) has shown for gelatin, and Meek and Gasser (2) for acacia.

The fluidity of concentrated solutions of acacia is of considerable advantage; equal concentrations of gelatin readily solidify upon cooling. On the other hand, gelatin has a much higher refractive index, thus permitting somewhat smaller injections than are used with acacia. Occasionally with acacia, never with gelatin, a tendency to vomit is noted in dogs, either during or shortly after injection. This nausea is of short duration and there are no observable bad after-effects. Usually such a reaction may be avoided by very slow injection (e.g., 5 cc. per minute).

TABLE 1  
*Refractive indices of gelatin and acacia solutions*

REFRACTIVE INDICES OF 1 PER CENT ACACIA	REFRACTIVE INDICES OF 1 PER CENT GELATIN	MIXTURE EQUAL PARTS OF 1 PER CENT ACACIA AND 1 PER CENT GELATIN
0.00132	0.00168	0.00148
0.00124	0.00160	0.00144
0.00132	0.00172	0.00152
0.00136	0.00172	
Average 0.00131	0.00168	0.00148
REFRACTIVE INDICES OF 5 PER CENT ACACIA	REFRACTIVE INDICES OF 5 PER CENT GELATIN	MIXTURE EQUAL PARTS OF 5 PER CENT ACACIA AND 5 PER CENT GELATIN
0.00655	0.00837	0.00738
0.00663	0.00833	0.00742
0.00655	0.00837	0.00742
0.00662	0.00845	
Average 0.0065875	0.00838	0.0074066+
$0.0065875/5=0.0013175$	$0.00838/5=0.001676$	$0.0074066+/5=0.0014813+$

Since it is sometimes advantageous to know the refractive indices of the stock solutions with which we work, we have spent some time in determining the values for diluted specimens from such solutions, and of less concentrated solutions made from the dry material. We have used commercial gum acacia, obtained in small lumps or "tears," containing an appreciable amount of foreign particles; also "Gold Label" sheet gelatin. It may very well be that chemically purified materials give somewhat different refractive indices from those which we have recorded. It will be observed that the refractive indices of 1 per cent solutions of acacia are about 0.00131 or 0.00132 greater than the solvent-distilled water, and that of gelatin is about 0.00167 or

0.00168, whether the readings are made on 1 per cent solutions or on 5 per cent readings and divided by 5. We also have determinations on 2 per cent and 10 per cent solutions which are comparable. It may be said that the readings as noted in table 1 are on separate solutions in each case, not different readings on the same solution. Our percentage solutions are made by putting the dry (desiccated) substance in a volumetric flask and making up to volume. In case of concentrated solutions (above 10 per cent at least) suitable dilutions are made to facilitate reading. It will be noted that the mixtures of equal parts 1 per cent acacia and 1 per cent gelatin give a value (0.00148 +) very close to the theoretical mean (0.001495 +) as found in the other determinations cited.

#### METHOD

The Pulfrich refractometer which reads the angle of total reflection to within one minute has been used for all this work. A sodium flame is used as the source of light. By means of a table the refractive indices corresponding to angles of total reflection are determined directly. Ordinarily distilled water is used as a basis for comparison. For instance, the refractive index of a 1 per cent aqueous solution of sodium chloride is 0.00160 greater than that of distilled water. The angles of total reflection of both water and salt solution as read will depend upon the temperature, the refractive index of the refractometer prism, etc., but under the same conditions of reading this difference of 0.00160 remains constant. In principle our method depends upon the difference in reading between the non-protein fraction of the blood serum before and again after the injection of a solution of a suitable colloid into the circulation.

It will be observed that in many of our experiments we have used a concentrated solution of acacia plus gelatin; that is, a solution of 20 per cent to 25 per cent acacia and 10 to 20 per cent gelatin in Locke's solution or physiological salt solution. Such a mixture is filtered through cotton, or centrifugalized to get rid of foreign material. Upon standing or centrifugalization two rather definite layers appear. The upper layer forms a soft gel upon cooling, while the lower layer never gels, though viscid, and is easily pipetted from underneath the firmer top portion. This fluid mixture we employ for injections. It remains homogeneous and has a high refractive index, the 1 to 20 dilutions reading well over 0.00200. The top layer may be warmed and injected if so desired, though we have been accustomed to use it as part of the next stock batch of gelatin-acacia mixture made up.

We usually inject (in dogs) about 1 cc. of concentrated solution per pound body weight. This amount is usually measured directly into one or more syringes. Accurate measurement of this thick, viscid fluid presents certain difficulties. The solutions are so viscous that they do not rapidly drain from a pipette. We rinse the pipette with a little warm saline solution, or draw the fluid a certain distance beyond the mark and drain only a short while. If the latter method is employed, one should calibrate the pipette for delivery at a given temperature, and should allow a constant period for drainage. Again it may be advisable to calibrate a large syringe and draw the solution up directly. If desired the solution may be warmed after measurement in the cold by laying the syringe over a steaming dish or radiator with a rubber band over the opening to prevent loss of fluid when expansion takes place.

At least three tubes should be at hand for blood samples; two of these should be clean centrifuge tubes, or test tubes, for the serum samples; and one an hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate solution for the plasma sample. A second hematocrit tube may be prepared for a plasma sample after injection, if so desired.

In dogs the blood is ordinarily withdrawn from the external jugular vein. We take from 5 to 10 cc. into a dry tube for a serum sample, and 7 to 10 cc. into oxalate for a plasma sample before injecting. The injection should be made slowly, about 5 to 10 cc. per minute, the syringe rinsed with warm saline, and the washings injected. If the dog has veins easy to enter it is just as well to withdraw the needle after the injection is completed, and use the opposite side for the second blood sample. In case the veins are small and difficult to find, the needle may be kept open by slow injection of salt solution, and the second serum sample obtained on the same side. We allow 3 to 5 minutes after the injection for mixing before taking the second sample for serum.

After standing a few minutes, the clotted samples are detached from the sides of the tubes and centrifugalized for about 10 minutes for separation of the clear serum. We allow about 30 minutes at 2500 revolutions per minute for the plasma hematocrit samples.

In determining the non-protein fraction of the serum we follow the method as outlined by Robertson (4) in which the serum proteins are precipitated by N/25 acetic acid. Glass tubes 20 to 25 cm. long, having an inside diameter of at least 5 mm. and walls about 1 mm. thick, are sealed at one end. It is well to blow gently into the tube while the

sealed end is still soft, thus rounding the bottom and lessening the tendency to crack on cooling. These inexpensive tubes may be used over again after cleaning, until they become too short. Into these tubes, which have been carefully cleaned and dried, are introduced definite amounts of serum by means of any small bored pipette with a capillary tip. If possible, duplicates of each serum sample should be made. We use approximately 1 cc. amounts, although as little as 0.4 or 0.5 cc. is sufficient so long as exactly the same volume of N/25 acetic acid is added. In delivering the serum one should avoid wetting the upper part of the tube, and the formation of air bubbles.

The serum having been delivered, the same amount of N/25 acetic acid as of serum is introduced into each tube. The same pipette as used for measuring the serum, after being rinsed with distilled water and N/25 acetic acid, or a second pipette calibrated against it is used to measure the acid. In case acacia solution has been the injection fluid, the tubes of serum after injection will show much more cloudiness than the first sample, but this entirely disappears on subsequent shaking. The N/25 acetic acid solution may be made up with sufficient accuracy by diluting 4 cc. glacial acetic acid to 1750 cc.

A small glass bead is next dropped into each tube and the open end is sealed off in a flame, care being taken not to heat the contents. After cooling, the tubes are reversed a sufficient number of times to insure a thorough admixture of the contents. The tubes are next placed in a beaker containing cold water of such a depth as to completely immerse the tops of the fluid columns. It is well to pad the bottom of the beaker with cotton to guard against cracks in bumping when the water boils. The water is slowly heated to boiling and allowed to boil for 2 minutes. The tubes are then removed from the boiling water and cooled to room temperature, either in water or more slowly in air.

When the tubes have cooled, the sealed tips are broken off. The coagulum is broken up by means of a small clean wire, after which the tubes are centrifugalized for a few minutes. The clear supernatant fluid representing the non-protein fraction of the blood serum can be easily decanted or pipetted off. The samples taken before and those taken after injection are next read against each other in the refractometer. It is well to have a constant temperature in the room where the readings are done. To obtain this it may be necessary to leave the flame which is used as the source of light burning in the room for an hour or more before reading. Otherwise, at least in a long series of determinations, it may be necessary to re-read a given fluid such as



water or salt solution at frequent intervals to find the change due to rising temperature. Between separate samples the cup of the refractometer should be rinsed with distilled water and carefully dried with absorbent cotton or filter paper, preferably followed by lens paper.

We consider that the actual reading of the refractometer is the simplest step in the whole method. With a little practice anyone can bisect the opposite angles of the X with the well demarcated line between light and shadow shifted by the fine adjustment screen. It is better for one individual to make all the readings in a given experiment, since possible slight errors are then more likely to offset each other. The angles as read on the scale are referred to a table giving the corresponding refractive indices, and the difference between solutions is obtained at once by subtraction.

The plasma percentage before injection is calculated from the hematocrit tube readings, allowance being made for the oxalate solution previously in the tube. The total plasma withdrawn before injection is subtracted from the total fluid volume injected; the result we assume to be the increment to the plasma volume caused by injection.

A suitable dilution (e.g., 1 to 20) is made of the concentrated injection fluid and its increased refractivity over the salt solution solvent is determined.

We now calculate:

$$\frac{\text{Amount Injected} \times \text{Reading of Diluted Sample} \times \text{Dilution}}{\text{Difference in Refractivity of Non-Proteins}} = \text{Increased Plasma Volume}$$

Plasma Volume Before Injection = Increased Plasma Volume - Plasma Increment.

$$\text{Blood Volume} = \frac{\text{Plasma Volume (Before Injection)}}{\text{Plasma Per Cent}} \times 100.$$

The difference in refractivity of non-proteins is obviously twice the difference in refractometer readings, since each serum sample is diluted one-half with N/25 acetic acid.

In case simple solutions of gelatin or acacia are used the plasma volume may be determined as follows: Knowing the reading of a true 1 per cent solution, and that of an accurately diluted sample of the concentrated injection fluid, the number of grams injected may be determined. From the difference in the non-protein reading before and after the injection the concentration per cubic centimeter of plasma

can be calculated. Then the grams injected divided by the plasma concentration minus the plasma increment should give the same value as arrived at in the above formula. In case an hematocrit is taken after injection the above figuring may be done without subtracting the plasma increment and the larger result divided by the plasma per cent after injection; the result is probably somewhere near the blood volume after injection. From this the total volume injected minus the total blood withdrawn may be subtracted, and the resulting figure is often very close to the blood volume before injection as determined by the first formula. However, we consider the first method to be the more reliable.

In these calculations we are considering the vascular tree as a definite container with impermeable walls, into which a known fluid is injected, the whole mixed, and the total contents determined by the concentration of injected material in a given sample of the diluted fluid. Obviously we are not justified in these assumptions; indeed it is occasionally found that the plasma percentage in the sample after injection is less than it was beforehand. No doubt there are changes occurring in the blood stream that are quite beyond our control and about many of which we know nothing. There have been observations in this laboratory which indicate a frequent leucopenia after injection of acacia; sometimes there seems to be a definite delay of clotting after injection of acacia although *in vitro* it requires practically an equal volume of 10 per cent acacia to prevent clotting. However, other blood volume methods meet the same or similar difficulties, in that changes going on *in vivo* are not fully understood.

#### EXPERIMENTAL OBSERVATIONS

It must be demonstrated at once that acacia and gelatin alone or combined are not carried out of the blood plasma by blood coagulation. It must be shown that these two colloids may be recovered quantitatively from the non-protein fraction of the blood serum. Our experiments have convinced us that acacia and gelatin are not disturbed by blood coagulation and do appear quantitatively in the non-protein fraction of the blood when this method is employed as described. Experiment 1 may be taken as a typical example.

*Experiment I. Recovery of acacia and gelatin in vitro.* The following solutions were prepared:

1. One cubic centimeter 10 per cent acacia in NaCl solution + 9 cc. whole blood.

2. One cubic centimeter NaCl solution + 9 cc. whole blood. (Control.)
3. One cubic centimeter approximately 10 per cent gelatin in NaCl solution + 9 cc. whole blood.
4. One cubic centimeter NaCl solution + 1 cc. gelatin and acacia mixture in NaCl solution + 8 cc. whole blood.
5. Two cubic centimeters NaCl solution + 8 cc. whole blood. (Control.)
6. One cubic centimeter 10 per cent acacia in NaCl solution + 9 cc. NaCl solution.
7. One cubic centimeter approximately 10 per cent gelatin in NaCl solution + 9 cc. NaCl solution.
8. One cubic centimeter gelatin and acacia mixture in NaCl solution + 19 cc. NaCl solution.
9. NaCl solution.

## Non-protein readings:

	<i>Angle of Ref.</i>	<i>Index</i>
1.....	$\begin{cases} 66^{\circ}6' \\ 66^{\circ}6' \end{cases}$	1.33855
2.....	$\begin{cases} 66^{\circ}26' \\ 66^{\circ}26' \end{cases}$	1.33695
3.....	$\begin{cases} 66^{\circ}4' \\ 66^{\circ}4' \end{cases}$	1.33871
4.....	$\begin{cases} 65^{\circ}50' \\ 65^{\circ}50' \end{cases}$	1.33983
5.....	$\begin{cases} 66^{\circ}26' \\ 66^{\circ}26' \end{cases}$	1.33695
Other solutions:		
6.....	$\begin{cases} 66^{\circ}4'30'' \\ 66^{\circ}4'30'' \end{cases}$	1.33867
7.....	$\begin{cases} 66^{\circ}3' \\ 66^{\circ}3' \end{cases}$	1.33879
8.....	$\begin{cases} 66^{\circ}6' \\ 66^{\circ}6' \end{cases}$	1.33855
9.....	$\begin{cases} 66^{\circ}21' \\ 66^{\circ}21' \end{cases}$	1.33735

Blood plasma percentage = 41.65 per cent.

$$1.33855 (1) - 1.33695 (2) = 0.00160; 0.00160 \times 2 = 0.00320.$$

$0.00320 \times 0.4165 = 0.0013328$ , reading of 1 per cent acacia in the whole blood.

$1.33867 (6) - 1.33735 (9) = 0.00132$ , reading of 1 per cent acacia in NaCl solution.

$$(100 \times 0.0013328 - 0.00132) / 0.00132 = 0.9 \text{ per cent difference.}$$

$$1.33871 (3) - 1.33695 (2) = 0.00176; 0.00176 \times 2 = 0.00352.$$

$0.00352 \times 0.4165 = 0.00146608$ , 1 per cent gelatin in whole blood.

$$1.33879 (7) - 1.33735 (9) = 0.00144, \text{ 1 per cent gelatin in NaCl solution.}$$

$$(100 \times 0.00146608 - 0.00144) / 0.00144 = 1.7 \text{ per cent difference.}$$

$$1.33983 (4) - 1.33695 (5) = 0.00288; 0.00288 \times 2 = 0.00576.$$

$0.00576 \times 0.4165 = 0.00239904$ , reading of 1:10 dilution of gelatin and acacia mixture in blood.

$$1.33855 (8) - 1.33735 (9) = 0.00120, \text{ reading of 1:20 dilution of mixture.}$$

$2 \times 0.00120 = 0.00240$ , reading of 1:10 dilution of gelatin and acacia mixture in NaCl solution.

$$(100 \times 0.00240 - 0.00239904) / 0.00240 = 0.04 \text{ per cent difference.}$$

It will be seen that within experimental error the recovery of acacia and gelatin was complete in whole blood. Where the gelatin and acacia were used separately a slight positive error occurred, and when a mixture of the two was employed the check was almost perfect.

For a considerable range of angles the index per minute is 8 in the 5th decimal place; hence the difference between two solutions is the difference in minutes  $\times 0.00008$ . As:

$$66^\circ 26' - 66^\circ 6' = 20'; 20 \times 0.00008 = 0.00160$$

In ordinary calculations we seldom use decimals in referring to refractive indices, thus for convenience we generally call that of acacia simply 132, and gelatin 168, etc.

It has been of interest to determine whether the method can be used with accuracy in the presence of hemolysis, jaundice, lipemia, etc. These conditions sometimes offer considerable difficulties in dye determinations of blood volume. We have found that these factors introduce no error in the refractometric method. Hemoglobin is entirely precipitated with the proteins. We have never taken any special precautions to prevent hemolysis, consequently a moderate grade has sometimes occurred. Such experiments have always been tabulated as normal, without question. Bile pigments and fat even in excessive amounts are inconsequential factors after the processes of dilution and precipitation.

The following experiment illustrates recovery in the presence of bile.

*Experiment II. Recovery of media in the presence of bile.* Sample: 100 cc. fresh blood + 5 cc. dog's bile.

The following solutions were prepared:

1. Nine cubic centimeters serum (containing bile) + 1 cc. 0.9 per cent NaCl solution.

2. Nine cubic centimeters serum (containing bile) + 1 cc. gelatin and acacia mixture in 0.9 per cent NaCl solution—(3 samples).

3. Nine cubic centimeters 0.9 per cent NaCl solution + 1 cc. gelatin + acacia mixture (2 samples).

4. Nine hundredths per cent NaCl solution.

Non-protein readings:

1.....	<i>Angle of Ref.</i> $\begin{cases} 66^{\circ}38'30'' \\ 66^{\circ}38' \end{cases}$	Average $66^{\circ}38\frac{1}{2}'$
2.....	$\begin{cases} 66^{\circ}11'30'' \\ 66^{\circ}11'30'' \\ 66^{\circ}11' \end{cases}$	Average $66^{\circ}11\frac{1}{2}'$
Difference (1 and 2) = $26\frac{1}{4}'$ ; $26\frac{1}{4} \times 8 \times 2 = 430\frac{1}{2}$ .		
Readings of media:		
3.....	<i>Angle of Ref.</i> $\begin{cases} 65^{\circ}27' \\ 65^{\circ}26'30'' \end{cases}$	Average $65^{\circ}26\frac{1}{2}'$
4.....	$66^{\circ}20'$	
Difference (3 and 4) = $53\frac{1}{4}'$ ; $53\frac{1}{4} \times 8 = 426$ .		
$100 \times 430\frac{1}{2} - 426 / 426 = 1.09$ per cent error.		

It will be seen at once that the difference in recovery in NaCl solution and in jaundiced serum lies well within the experimental error of measurement and reading, and that even so, the error as noted is in favor of the serum mixture.

The following two experiments were performed upon the same dog, one during an induced lipemia, the other in a normal period. We have another experiment on the same animal on still another date which gives practically the same blood volume which these experiments show.

*Experiment III. Blood volume in lipemia. August 13, 1919.*

Dog 20-6. Young male adult, in good condition. Weight  $23\frac{1}{2}$  pounds (10.52 kgm.). Gave 50 cc. cottonseed oil + 100 cc. milk by stomach tube  $1\frac{1}{2}$  hours before withdrawal of blood.

Removed 16 cc. blood before injection. Plasma per cent = 57.

Injected  $24\frac{1}{2}$  cc. media + 20 cc. NaCl solution. Removed 18 cc. after 6 to 7 minutes.

Serum shows marked lipemia.

Media diluted 1:20 — reading  $66^{\circ}2'$ .....18' difference

NaCl solution — reading  $66^{\circ}20'$ ..... $18 \times 8 = 144$

Serum non-protein, before injection — reading..... $\begin{cases} 66^{\circ}19\frac{1}{2}' \\ 66^{\circ}19\frac{1}{2}' \end{cases}$

Serum non-protein after injection — reading.....  $\left\{ \begin{array}{l} 66^{\circ}13\frac{1}{2}' \\ 66^{\circ}13\frac{1}{2}' \end{array} \right.$

Difference =  $6\frac{1}{2}'$ ;  $6\frac{1}{2} \times 8 \times 2 = 100$ .

44.5 cc. total injection

9.0 cc. plasma withdrawn

35.5 cc. plasma increment

$$\frac{144 \times 24\frac{1}{2} \times 20}{100} - 35.5 = 670 \text{ cc. plasma volume.}$$

$670 / 0.57 = 1176 \text{ cc. blood volume.}$

$1176 / 10520 = 11.1 \text{ cc. per 100 grams body weight.}$

*Experiment IV. August 27, 1919.*

Dog 20-6. Young male adult (see exper. III). Weight 24 pounds (10.9 kgm.).

Removed 16 cc. blood before injection. Plasma = 54.7 per cent.

Injected 25 cc. acacia + gelatin mixture (previously found to read 164 in a 1:25 dilution) + 14 cc. NaCl solution.

Dog became a little sick but did not vomit.

Second blood sample withdrawn 4 - 5 minutes after injection.

1. Non-protein—before injection.....  $\left\{ \begin{array}{l} \text{Angle of Ref.} \\ 66^{\circ}19'30'' \\ 66^{\circ}19'30'' \\ 66^{\circ}20' \end{array} \right.$

2. Non-protein—after injection.....  $\left\{ \begin{array}{l} 66^{\circ}11' \\ 66^{\circ}11' \\ 66^{\circ}11' + \\ 66^{\circ}11' + \end{array} \right.$

*Angle of Ref.*

No. 1 (before injection) re-read because of rising temperature..  $66^{\circ}21'$

Difference about  $9\frac{1}{2}$  ( $66^{\circ}20\frac{1}{2}' - 66^{\circ}11'$ )

$$9\frac{1}{2} \times 8 \times 2 = 152$$

39 cc. total injection — 9 cc. plasma withdrawn = 30 cc. plasma increment.

$$\frac{164 \times 25 \times 25}{152} - 30 = 674 \text{ cc. plasma volume.}$$

$674 / 0.547 = 1177 \text{ cc. blood volume.}$

$1177 / 10900 = 10.7 \text{ cc. per 100 grams body weight.}$

That the blood volumes as determined in experiments III and IV so nearly coincide is a mere chance, especially when the time interval between determinations, the change in weight, etc., are considered. Nevertheless, the results certainly suggest that even a marked lipemia does not affect the non-protein readings. ■

With the possibility of error from these more or less abnormal conditions eliminated, we may go on to the consideration of normal cases.

Table 2 gives the results of blood volume determinations in a number of dogs. It is scarcely necessary to give protocols of the individual experiments; the essential details are given in the table, and experiments III and IV are indicative of the usual procedure and calculations.

Most of these dogs were in good condition, a few were very anemic as noted in the remarks.

In nearly every case we have dye determinations on the animal; some of these were done in the regular course of experiments by various laboratory workers, others were kindly performed by either Mr. H. P. Smith or Mrs. Robscheit, especially to serve as checks for our own experiments.

Blood volumes done by the dye method at an interval of several days or weeks from the refractometric method are of little comparative worth; the determinations done on the same day are of the greatest value. Sometimes the difference between the two results is rather large, but on the other hand, the similarity is often quite striking. We were pleased to find that the refractometric method sometimes gives higher results, sometimes lower than the dye method. This lack of constancy probably indicates the occurrence of experimental errors rather than a fundamental fault in the method. The ratio of blood volume to body weight is quite similar in the two methods. The average amount of blood per 100 grams body weight in the twenty-one experiments tabulated in table 2 is 9.76 cc.

In a series of dogs reported by Meek and Gasser (2) using the acacia-phloro-glucid method, the blood volume in per cent of body weight was 9.74 per cent. To compare with this, our results as given should be multiplied by the specific gravity of blood, but even so the similarity is rather striking. The plasma percentages of body weight in dogs as determined by Keith, Rowntree and Geraghty (1) are quite comparable to our results.

Reference to table 2 shows that we gave much larger injections in the early experiments than we did later. The later work was largely done with the solutions of high refractive indices. Furthermore, we came to the conclusion that large volumes of fairly concentrated colloids produced greater upset of equilibrium (i.e., changes in cell content and ratio, unaccountable plasma percentages, disturbances in protein content, etc.) than did small injections, and were to be avoided when possible. We attempted to obtain the blood volume in a few instances when Mr. Smith and Mr. Arnold injected very large amounts of 6 per cent acacia without previously producing an anemia. In two experi-

TABLE 2  
Blood volume results—refractometric method—dogs

EXPERIMENT NUMBER	DOG NUMBER	DATE	WEIGHT kgm.	INJECTION	PLASMA BEFORE INJECTION per cent	PLASMA VOLUME cc.	BLOOD VOLUME cc.	BLOOD PER 100 GRAMS BODY WEIGHT cc.	BLOOD VOLUME DYE METHOD	REMARKS
V	18-114	7/25/18	11.64	60 cc. 20 per cent acacia	49	611	1247	10.7		See next experiment
VI	18-114	8/3/18	11.50	60 cc. 20 per cent acacia	44.4	547	1232	10.7	1294	Dye determination made August 14
VII	18-113	7/25/18	12.44	60 cc. 20 per cent acacia	46.8	491	1049	8.4	940 (1) 1136 (2)	Dye determinations made (1) June 10, and (2) August 14
VIII	18-115	8/1/18	7	50 cc. 20 per cent acacia	60.58	461	760	10.8	761 (1) 1000 (2)	Dog is in poor condition. Dye determinations (1) June 10, and (2) September 19
IX	18-24	3/12/19	13.6	54½ cc. 20 per cent acacia		582	1294	9.5	1305	Calculated volume after injection. Dye determination also after injection
X	18-39	3/14/19	11.1	45 cc. 20 per cent acacia		553	1270	11.4	1255 (1) 1205 (2)	Calculated volume after injection Dye determinations (1) before and (2) after injection
XI	18-4	3/18/19	20.5	86 cc. 20 per cent acacia	37.1	804	2167	10.5	2264 (1) 2117 (2)	Dye determinations (1) before and (2) after injection of acacia



		19-11	4/15/19	20	64 cc. 25 per cent acacia	47	854	1817	9.1	1955 (1) 2630 (2)	Dye determinations (1) before and (2) after injection of acacia
XII											
XIII		17-157	8/8/19	10.02	34½ cc. 20 per cent acacia	50	514	1028	10.2	1123	Dye determination made on a different day, same week
XIV		18-38	7/7/19	10.68	34 cc. mixture gel- atin and acacia	48.21	464	962	9.0	903	Dye injected in acacia solution
XV		18-38	8/4/19	10.45	25 cc. mixture gelatin + acacia	50.8	513	1009	9.5		See previous experi- ment
XVI		16-160	8/9/19	12.045	24½ cc. mixture gelatin + acacia	45.2	540	1323	10.9	1040	Dye determination made August 18
XVII		18-126	8/12/19	10.795	24½ cc. approxi- mately 16 per cent gelatin	46.9	556	1185	10.9	975	Dye determination made August 18
XVIII		19-93	8/22/19	12.727	25 cc. mixture gel- atin + acacia	68.5	795	1160	9.1	1023	Dog anemic. Dye de- termination made on previous day
XIX		19-94	8/22/19	13.80	25 cc. mixture gel- atin + acacia	70	909	1298	9.4	1080	Dog anemic. Dye de- termination made on previous day
XX		19-95	8/23/19	13.98	25½ cc. mixture gelatin + acacia	63	718	1139	8.1	1108	Dog anemic. Dye de- termination made two days previously
XXI		19-96	8/23/19	14.204	25½ cc. mixture gelatin + acacia	61.2	642	1049	7.3	993	Dog anemic. Dye de- termination made two days previously

TABLE 2—Continued

EXPERIMENT NUMBER	DOG NUMBER	DATE	WEIGHT <i>kgm.</i>	INJECTION	PLASMA BEFORE INJECTION <i>per cent</i>	PLASMA VOLUME <i>cc.</i>	BLOOD VOLUME <i>cc.</i>	BLOOD PER 100 GRAMS BODY WEIGHT <i>cc.</i>	BLOOD VOL- UME DYE METHOD	REMARKS
XXII	19-84	8/25/19	9.432	20 cc. mixture gel- atin + acacia	41.7	434	1040	11.0	996	Dye injected with acacia-gelatin mix- ture
XXIII	19-83	8/25/19	14.9	25 cc. mixture gel- atin + acacia	44.82	665	1484	9.96	1545	Dye injected with acacia-gelatin mix- ture
XXIV	16-158	8/25/19	12.1	25 cc. mixture gel- atin + acacia	72.5	639	882	7.3	929	Dye injected with acacia-gelatin mix- ture
XXV	18-114	8/27/19	13.8	25 cc. mixture gel- atin + acacia	53.1	829	1561	11.3	1480	Dye injected with acacia-gelatin mix- ture
Average.....								9.76		

ments out of about half a dozen we obtained results comparable with the dye method. Such enormous injections are certainly unnecessary and unfavorable for subsequent refractometric determinations. Experiment XXVI shows one of the few good results which we obtained.

*Experiment XXVI. Blood volume determined following the injection of a large amount of 6 per cent acacia solution. August 8, 1919.*

Dog 17-38. Weight 28 pounds (12.72 kgm.).

Mr. Arnold injected 635 cc. of approximately 6 per cent acacia; this was the estimated plasma volume.

Sample following injection was removed in 5 minutes.

A 1:6 dilution of the injection media read 120.

Plasma per cent = 42.5 immediately preceding injection.

Difference in non-protein readings before and after =  $22\frac{1}{2}$ ;  $22\frac{1}{2} \times 8 \times 2 = 360$ .

$$\frac{120 \times 6 \times 635}{360} - 635 = 635 \text{ plasma volume before injection.}$$

$635 / 0.425 = 1496$  cc. blood volume.

Mr. Arnold obtained the following results by the dye method:

	Plasma volume	Blood volume
Before injection.....	585	1416
After injection.....	901	1567

In addition to experiments on dogs we have a few observations on rabbits.

With these animals we use an improvised box holder with side holes through which the ears are drawn. Blood is obtained from the marginal ear vein through a small longitudinal slit made with a safety razor blade. Collection is made into small tubes, one for plasma and one for serum, as with dogs. Three cubic centimeter hematocit tubes are made from sections of straight-sided, graduated 10 cc. pipettes; one end of a section is sealed off, and the graduations subsequently verified or modified if necessary. To prevent clotting 0.4 or 0.5 cc. of 1.6 per cent oxalate solution is used per tube. To dilate vessels and hasten bleeding an electric light bulb held almost touching the ear is found very satisfactory. Injection of the colloidal material is made into any available ear vein from a syringe with a small needle, and collection of blood sample after injection made from the marginal vein of the opposite ear. Using this technique little blood need be taken, and many determinations may be made upon the same animal with minimal injury. Table 3 gives the results on four rabbits.

The amounts of solution injected have always been quite small and no untoward reactions have been noted. The checks with dye determinations differ by 4.8 per cent to 11 per cent.

TABLE 3  
*Blood volume results on rabbits—refractometric method*

EXPERIMENT NUMBER	RABBIT NUMBER	DATE	WEIGHT grams	INJECTION	PLASMA BEFORE INJECTION per cent	PLASMA VOLUME cc.	BLOOD VOLUME cc.	BLOOD PER 100 GRAMS BODY WEIGHT grams	BLOOD VOLUME DYE METHOD	REMARKS
XXVII	I	7/23/19	3375	10 cc. 20 per cent acacia	60	149	248	7.3	280	Samples removed during period 8 to 17 minutes after injection. Dye de-termination made on same day
XXVIII	I	7/29/19	3375	9.5 cc. 20 per cent gelatin	64.8	159	245	7.2		Sample removed 10 minutes after injection
XXIX	I	8/7/19	3300	5 cc. concentrated mixture gelatin + acacia + 5 cc. 0.9 per cent NaCl solution	60.5	125	206	6.2		Sample removed 5 minutes after injection
XXX	I	8/24/19	3385	5 cc. concentrated mixture gelatin + acacia + 5 cc. 0.9 per cent NaCl solution	62.7	145	231	6.8		Sample removed 5 minutes after injection
XXXI	II	7/24/19	3100	10 cc. mixture gelatin + acacia	65.66	154	250	8.06	220 (1) 226 (2)	Sample removed 30 minutes after injection. Dye de-terminations made on same day (1) with ear veins (2) femoral vein and artery

XXXII	II	8/14/19	3150	10 cc. mixture gelatin + acacia	54.7	110 (1)	201 (1)	6.3		(1) Sample removed 5 minutes after injection
						122 (2)	223 (2)			(2) Sample removed 30 minutes after injection
XXXIII	III	7/21/19	3250	10 cc. mixture gelatin + acacia	62.8	144	229	7.0	244	Sample removed about 15 minutes after injection
XXXIV	III	8/5/19	3190	10 cc. mixture gelatin + acacia	58.3	112	193	6.05		Sample removed 5 minutes after injection
XXXV	III	8/15/19	3175	10 cc. mixture gelatin + acacia	59	118	200	6.3		Sample removed 5 minutes after injection
XXXVI	IV	7/22/19	2750	8 cc. mixture gelatin + acacia	57.7	130	225	8.1	200	Dye injected with gelatin-acacia mixture. Sample removed about 15 minutes after injection
XXXVI	IV	7/30/19	2650	4 cc. concentrated gelatin + acacia mixture + 6 cc. 0.9 per cent NaCl solution	62.6	136	217	8.1		Sample removed 30 minutes after injection
XXXVIII	IV	8/9/19	2800	10 cc. mixture gelatin + acacia	60.3	113	188	7.2		Sample removed 5 minutes after injection
XXXIX	IV	8/18/19	2850	10 cc. mixture gelatin + acacia	57	119	209	7.8		Sample removed 15 minutes after injection
XL	IV	8/30/19	2550	7.5 cc. 20 per cent acacia	58.1	97	167	6.6		Sample removed 5 minutes after injection
Average.....										7.07

The large ratio of blood volume to weight is rather interesting. We think that without doubt the blood volume as determined by samples obtained 5 minutes after injection should be considered the most accurate. Averaging the values obtained from these 5-minute samples we find that the average of 7.07 cc. of blood per 100 grams body weight is reduced to 6.49. Even this is much higher than the values usually accepted. Meek and Gasser (2) give an average of 5.44 per cent of the body weight, and other observers even less.

We have several experiments on rabbits where blood samples were withdrawn at varying intervals, which show increasing blood volumes as time goes on. This, of course, is due to at least two factors: *a*, the actual elimination of acacia, and *b*, the dilution of the plasma, presumably by fluid drawn into the blood stream from the tissues because of increased osmotic pressure. The following abbreviated protocol of experiment XXXV shows the changes mentioned.

*Experiment XXXV. Blood volume. August 15, 1919.*

Rabbit III. Weight 3175 grams.

Injected 10 cc. gelatin + acacia mixture (reading 1:12½ dilution = 0.00172) and 3 cc. of 0.9 per cent solution NaCl.

I. Sample before injection. Plasma per cent = 59. (Removed 4.35 cc. blood). Non-protein readings  $\begin{cases} 66^{\circ}25' \\ 66^{\circ}25' \end{cases}$

II. Sample removed 5 minutes after injection. Non-protein readings  $\begin{cases} 66^{\circ}15\frac{1}{2}' \\ 66^{\circ}15\frac{1}{2}' \end{cases}$

III. Sample removed 15 minutes after injection. Non-protein readings  $\begin{cases} 66^{\circ}16' \\ 66^{\circ}16' \end{cases}$

IV. Sample removed 30 minutes after injection. Plasma per cent = 61. Non-protein readings  $\begin{cases} 66^{\circ}17\frac{1}{2}' \\ 66^{\circ}17\frac{1}{2}' \end{cases}$

V. Sample removed 1 hour after injection. Plasma per cent = 63. Non-protein readings  $\begin{cases} 66^{\circ}18\frac{1}{2}' \\ 66^{\circ}18\frac{1}{2}' \end{cases}$

VI. Sample removed 20 hours after injection. Plasma per cent = 61.7. Non-protein readings  $\begin{cases} 66^{\circ}22' \\ 66^{\circ}22' \end{cases}$

Altogether, 23.27 cc. blood were removed in 10 samples.

Calculating as in previous experiments the results are approximately:

SAMPLE	PLASMA VOLUME	BLOOD VOLUME	PER 100 GRAMS BODY WEIGHT
5 minutes.....	118	200	6.3
15 minutes.....	124	210	6.6
30 minutes.....	148	241	7.5
1 hour.....	169	268	8.4

It will be noted that the plasma percentage increased for at least an hour. Such variations in values with lapse of time inclines us to accept the results obtained from 5-minute samples. This probably gives enough time for mixing and a minimal time for elimination and dilution. The reading of the sample after 20 hours indicates that an appreciable amount of injection media is still in the circulation. We have numerous observations of a similar nature showing that total elimination is slow, and corroborating the results of previous workers.

We have done very little work with cats because of a scarcity of animals. Our technique has been to inject into the femoral vein, and withdraw blood from the femoral artery through a cannula. Our experiments are so few in number that we consider it unwise to report these experiments at this time.

#### DISCUSSION

The colloid-refractometric blood volume method as outlined gives results quite comparable to the dye methods, and to Meek and Gasser's acacia method. This method requires a slightly longer time to complete than the dye method but it is much shorter than the method of Meek and Gasser. A routine determination can be finished in about two hours.

We have considered reading the plasma or serum difference without precipitating the proteins, but the dilution of the protein content makes such determination altogether unreliable. An alternate possibility, however, is to use the plasma non-proteins, which necessitates only one blood sample before injection and one afterward. A serious drawback to such a procedure is that the samples put into oxalate solution must be measured and of exactly equal volume in order that one may be read against the other; also the dilution with oxalate must be considered in computations. However, this method can be employed in conditions in which the blood for any reason fails to clot or clots incompletely.

We have tried double blood volume determinations, that is, on the basis of two separate injections at short intervals, only twice (on rabbits) and then without success. The recovery after the second injection was less than was to be expected in these instances. We can offer no explanation of this failure; probably further work with dogs as well as rabbits will clear up the difficulty.

If Meek and Gasser (2) can recover acacia quantitatively as phloroglucid after a second injection, presumably there should be no difficulty in recognizing it refractometrically.

It has occurred to us that the transient white cloudy precipitate formed upon adding dilute acetic acid to blood serum containing acacia might be used as a qualitative method in studying the elimination curve of acacia from the blood stream.

The injection media and materials used in this method are comparatively cheap and are always available. To laboratories equipped with a refractometer we offer the procedure as worthy of trial. The time needed for a complete determination is not too long for ordinary work, the individual steps are relatively simple, and the results compare favorably with those obtained by other methods. A point of considerable advantage is the small amount of blood needed for the determinations; if necessary, accurate results should be obtained with one cubic centimeter of serum before, and one after injection, and a micro-hematocrit reading. Another point of value in the method is that hemolysis, lipemia and cholemia are not disturbing factors.

#### SUMMARY

We have outlined a method of determining blood volume which consists essentially in reading refractometrically the serum non-protein increase after the intravenous injection of a known amount of acacia or gelatin solution, or a mixture of the two.

By this method we have found in the dog an average of 9.76 cc. blood per 100 grams body weight. In the rabbit an average of determinations using samples removed 5 minutes after injection of media gives a volume of 6.49 cc. per 100 grams body weight.

Of great importance is the fact that hemolysis, lipemia and cholemia do not affect the accuracy of determinations by this method.

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## THE INFLUENCE OF SPLENIC EXTRACT ON THE NUMBER OF CORPUSCLES IN THE CIRCULATING BLOOD

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These experiments were undertaken in order to determine the effect on the number of both red and white corpuscles in the circulating blood of injections of splenic extract. It is believed that such a determination must throw some light on the possibility of the presence of an internal secretion of the spleen capable of affecting formation or destruction of blood corpuscles.

For reasons stated elsewhere (1) rabbits were used in all cases and, to exclude so far as possible changes in the corpuscle content of the blood accompanying digestion, the animal being used was deprived of food and water during the course of the experiment. The procedure followed was to make an initial count of both red and white corpuscles and then to inject subcutaneously the desired dose of splenic preparation. The dose was dissolved in physiological saline solution and warmed to 37°C. before injection. Subsequent red and white corpuscle counts were made at half-hour intervals during the first two or three hours after injection and a final count was made four or five hours from the beginning of the experiment. The diluting fluids used in making the corpuscle counts were 0.7 per cent sodium chloride solution for the erythrocytes and 0.5 per cent acetic acid for the leucocytes. The counts were made in the usual manner with Levy haemacytometers.

In the first series of experiments the preparation employed was powdered dried spleen, prepared by Armour and Company, one gram of which is said to represent approximately five grams of fresh spleen of the calf. The dose used was 10 mgm. of this powder per kilogram of body weight of the rabbit, dissolved in about 2 cc. of physiological saline solution and injected subcutaneously. The protocol of a typical experiment of this group follows:

*Experiment 2, November 3, 1918*

- 10.42 a.m. Red blood corpuscles 7,264,000 per cubic millimeter. White blood corpuscles 9,200 per cubic millimeter.
- 10.45 a.m. 10 mgm. powdered dried spleen (Armour and Company) per kilogram of body weight injected subcutaneously.
- 11.15 a.m. Red blood corpuscles 6,800,000 per cubic millimeter. White blood corpuscles 7,000 per cubic millimeter.
- 11.45 a.m. Red blood corpuscles 6,440,000 per cubic millimeter. White blood corpuscles 7,000 per cubic millimeter.
- 12.15 p.m. Red blood corpuscles 6,704,000 per cubic millimeter. White blood corpuscles 6,200 per cubic millimeter.
- 12.45 p.m. Red blood corpuscles 6,944,000 per cubic millimeter. White blood corpuscles 7,200 per cubic millimeter.
- 2.45 p.m. Red blood corpuscles 6,976,000 per cubic millimeter. White blood corpuscles 14,100 per cubic millimeter.

TABLE 1

*Decrease in erythrocyte count after injection of powdered dried spleen (Armour and Company), 10 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MINIMAL COUNT	AMOUNT OF DECREASE	PERCENT- AGE EFFECT	MINIMUM IN	DURATION OF EFFECT
					minutes	minutes
1	6,424,000	6,352,000	72,000	1.12	30	30
2	7,264,000	6,440,000	824,000	11.34	60	90
3	7,168,000	6,336,000	832,000	11.60	90	120
4	6,624,000	6,256,000	368,000	5.55	30	60
5	7,104,000	6,608,000	496,000	6.98	30	90
6	6,144,000	6,080,000	64,000	1.04	60	90
7	6,960,000	6,160,000	808,000	12.93	30	60
8	7,175,000	6,960,000	215,000	2.99	30	60
9	7,488,000	7,144,000	344,000	4.59	30	30
10	6,424,000	6,376,000	48,000	0.74	60	60
Averages	6,877,500	6,471,200	406,300	5.88	45	69

There are ten experiments in this group, in each of which the result was practically the same—a slight reduction in the red corpuscle count of short duration (table 1), a prompt reduction in the number of white corpuscles followed in several hours by a considerable leucocytosis. The change in the leucocyte count is identical with the effect described by Wells (2) and others as following upon the subcutaneous injection of any foreign protein and can not, therefore, be considered a specific effect of spleen unless it is still obtained after the administration of a protein-free preparation.

We attempted to obtain a protein-free splenic preparation by precipitation with alcohol. A 2.5 per cent solution in distilled water of the powdered dried spleen was made and poured in a thin stream into absolute alcohol. The proportion of solution to alcohol used was so arranged that the resulting mixture always contained at least 80 per cent of alcohol. The precipitate thus formed was filtered off and the alcoholic filtrate evaporated to dryness on a water bath. Five grams of the powdered spleen yielded 1.5 grams of final residue or about 60 mgm. per gram of fresh spleen. While realizing that this residue is very probably not rendered absolutely protein free by the method employed, we will for convenience speak of it hereafter as protein-free splenic preparation (Armour and Company). In the second series of experiments this residue was used in the dose of 10 mgm. per kilogram of body weight of the rabbit. The protocol of one of the experiments of this group follows:

*Experiment 14, January 27, 1919*

- 10.20 a.m. Red blood corpuscles 6,960,000 per cubic millimeter. White blood corpuscles 7,000 per cubic millimeter.
- 10.25 a.m. 10 mgm. protein-free splenic preparation (Armour and Company) per kilogram of body weight injected subcutaneously.
- 10.55 a.m. Red blood corpuscles 5,712,000 per cubic millimeter. White blood corpuscles 8,400 per cubic millimeter.
- 11.25 a.m. Red blood corpuscles 5,632,000 per cubic millimeter. White blood corpuscles 7,000 per cubic millimeter.
- 11.55 a.m. Red blood corpuscles 5,816,000 per cubic millimeter. White blood corpuscles 8,500 per cubic millimeter.
- 12.25 p.m. Red blood corpuscles 6,624,000 per cubic millimeter. White blood corpuscles 11,600 per cubic millimeter.
- 12.55 p.m. Red blood corpuscles 6,816,000 per cubic millimeter. White blood corpuscles 8,200 per cubic millimeter.
- 2.55 p.m. Red blood corpuscles 7,200,000 per cubic millimeter. White blood corpuscles 6,800 per cubic millimeter.

Again we find that the red corpuscle count decreased following the injection (table 2), the decrease in these experiments being much more marked and more persistent than in the first series. The characteristic effect of foreign protein injections on the white corpuscles is no longer in evidence. On the other hand, an increased leucocyte count of brief duration is recorded in every experiment, occurring at or about the time of maximum decrease of the red corpuscles (table 3). We feel justified, therefore, in concluding that the effect on the erythrocyte count at least is a specific effect of the splenic preparation employed.

We now proceeded to make fresh extracts of the spleen of dogs and cats. The fresh spleen was ground with sand and macerated over night with twice its bulk of physiological saline solution. The prepara-

TABLE 2

*Decrease in erythrocyte count after injection of protein-free splenic preparation (Armour and Company), 10 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MINIMAL COUNT	AMOUNT OF DECREASE	PERCENT- AGE EFFECT	MINIMUM IN	DURATION OF EFFECT
					minutes	minutes
11	6,336,000	4,672,000	1,664,000	26.26	90	150
12	6,672,000	5,664,000	1,008,000	15.10	60	90
13	7,328,000	6,480,000	848,000	11.57	60	90
14	6,960,000	5,632,000	1,328,000	19.08	60	120
15	6,800,000	4,992,000	1,080,000	26.58	90	120
16	7,232,000	6,248,000	984,000	13.60	60	90
17	6,272,000	4,384,000	1,888,000	30.10	60	90
18	5,360,000	4,800,000	560,000	10.44	60	90
19	6,940,000	5,456,000	1,484,000	21.38	60	120
20	6,456,000	5,632,000	824,000	12.76	60	60
Averages	6,635,600	5,396,000	1,239,600	18.68	66	102

TABLE 3

*Increase in leucocyte count after injection of protein-free splenic preparation (Armour and Company), 10 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMAL COUNT	AMOUNT OF INCREASE	PERCENTAGE EFFECT	MAXIMUM IN
					minutes
11	6,400	10,500	4,100	64.06	150
12	8,800	13,600	4,800	54.54	120
13	11,000	15,200	4,200	38.18	60
14	7,000	11,600	4,600	65.71	120
15	6,200	15,500	9,300	150.00	60
16	13,300	16,600	3,300	24.06	60
17	11,100	13,000	1,900	17.11	90
18	10,900	12,800	1,900	17.43	90
19	12,000	15,000	3,000	25.00	30
20	11,500	14,800	3,300	28.69	30
Averages.....	9,820	13,860	4,040	41.14	81

tion was then strained through muslin and filtered. The filtrate was poured in a thin stream into absolute alcohol, care again being taken not to reduce the alcohol below 80 per cent. The precipitate was

filtered off and the alcoholic extract evaporated to dryness on the water bath. We obtained from dog's spleen about 26 mgm. of final residue per gram of fresh spleen and from cat's spleen about 10 mgm. per gram of fresh spleen.

Five experiments were performed in each of which 10 mgm. of the extract of dog's spleen per kilogram of body weight were injected subcutaneously, and two experiments in each of which a similar dose of the extract of cat's spleen was administered. The results so far as the red corpuscles are concerned are shown in tables 4 and 5.

TABLE 4

*Decrease in erythrocyte count after injection of extract of dog's spleen, 10 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MINIMAL COUNT	AMOUNT OF DECREASE	PERCENT- AGE EFFECT	MINIMUM IN	DURATION OF EFFECT
					minutes	minutes
21	7,216,000	5,520,000	1,696,000	23.50	30	150
22	6,976,000	5,900,000	1,076,000	15.42	120	150
23	6,656,000	4,561,000	2,095,000	31.49	60	150
24	6,000,000	5,152,000	848,000	14.10	30	120
25	5,952,000	4,592,000	1,360,000	22.84	30	150
Averages	6,560,000	5,145,000	1,415,000	21.47	54	144

TABLE 5

*Decrease in erythrocyte count after injection of extract of cat's spleen, 10 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MINIMAL COUNT	AMOUNT OF DECREASE	PERCENT- AGE EFFECT	MINIMUM IN	DURATION OF EFFECT
					minutes	minutes
26	6,432,000	5,376,000	1,056,000	16.41	30	90
27	6,136,000	5,400,000	736,000	11.99	30	60
Averages	6,284,000	5,388,000	896,000	14.20	30	75

In both groups of experiments there occurred a decrease in the red corpuscle count similar to that described following the administration of the splenic preparations (Armour and Company). Furthermore, in the experiments with extract of dog's spleen a transient increase in the leucocytes appeared at about the time the red corpuscles were decreased to the greatest extent (table 6). Such a change in the white corpuscle count was not observed, however, after the injection of extract of cat's spleen.

TABLE 6

*Increase in the leucocyte count after injection of extract of dog's spleen, 10 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMAL COUNT	AMOUNT OF INCREASE	PERCENTAGE EFFECT	MAXIMUM IN
					minutes
21	11,000	11,800	800	7.27	90
22	14,000	19,000	5,000	35.71	30
23	12,000	18,000	6,000	50.00	120
24	12,200	14,000	1,800	14.75	90
25	8,000	10,400	2,400	30.00	30
Averages.....	11,440	14,640	3,200	27.54	72

TABLE 7

*Decrease in erythrocyte count after injection of extract of dog's spleen, 20 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MINIMAL COUNT	AMOUNT OF DECREASE	PERCENT- AGE EFFECT	MINIMUM IN	DURATION OF EFFECT
					minutes	minutes
28	6,678,000	5,320,000	1,358,000	20.33	90	210
29	7,336,000	5,312,000	2,024,000	27.58	60	150
30	9,632,000	5,888,000	3,744,000	38.87	120	150
31	7,216,000	4,304,000	2,912,000	40.35	120	150
32	6,864,000	4,944,000	1,920,000	27.97	60	150
Averages.	7,545,200	5,153,600	2,391,600	31.02	90	162

TABLE 8

*Decrease in erythrocyte count after injection of extract of dog's spleen, 40 mgm. per kilogram of body weight*

EXPERIMENT NUMBER	INITIAL COUNT	MINIMAL COUNT	AMOUNT OF DECREASE	PERCENT- AGE EFFECT	MINIMUM IN	DURATION OF EFFECT
					minutes	minutes
33	5,072,000	2,560,000	2,512,000	49.52	30	120
34	7,644,000	6,272,000	1,372,000	17.94	60	120
35	6,704,000	5,492,000	1,212,000	18.07	90	150
36	6,256,000	4,512,000	1,744,000	27.87	90	150
37	8,720,000	7,344,000	1,376,000	15.77	120	150
Averages.	6,879,200	5,236,000	1,643,200	23.88	74	134

These results establish the presence in the splenic preparations of an agent capable of reducing by some means the number of red corpuscles in the circulating blood. Is this effect quantitative, that is, can it be increased by increasing the dose of the splenic preparation? To determine this point ten additional experiments were carried out. In five of these 20 mgm. of the extract of dog's spleen per kilogram of body weight were injected subcutaneously. In the other five experiments 40 mgm. of the same preparation per kilogram of body weight were injected. The results of these injections on the number of red corpuscles are shown in tables 7 and 8.

The dose of 10 mgm. of extract of dog's spleen per kilogram of body weight decreased the erythrocyte count 21.47 per cent; the dose of 20 mgm. per kilogram decreased the count 31.02 per cent; the dose of 40 mgm. per kilogram decreased the count 23.88 per cent. It would seem from this that the effect is not entirely progressive, but further consideration will be given to this point later.

If the decrease in the number of red corpuscles in the circulating blood is the result of a direct destructive action exerted by the splenic agent on the corpuscles, it should be possible to cause their destruction by bringing them into contact with this agent outside of the body. We tried to determine whether this could be done by the procedure now to be described. Specimens of blood from an ear vessel of a rabbit were obtained simultaneously in two haemocytometer pipettes in the usual manner. The blood in one pipette was then diluted with 0.7 per cent sodium chloride solution while the blood in the other was diluted with 0.7 per cent sodium chloride solution in which was dissolved extract of dog's spleen in definite amount. Several successive drops of diluted blood from each pipette were counted and the counts from each averaged and compared. In order to have a check on any difference in their accuracy the pipettes and counting chambers after thorough cleansing were alternated and new specimens of blood from the same animal secured and counted. Counts made in this way showed close agreement.

According to Meek and Gasser (3) the average volume of the blood in the rabbit is 100 cc. (5 per cent of body weight). Assuming complete absorption after the subcutaneous injections the maximum amount of splenic extract which could be present in the blood at any time would be about 20, 40 or 80 mgm. per 100 cc. of blood for the doses of 10, 20 or 40 mgm. per kilogram of body weight. On this basis we made determinations using dilutions of 20, 40, 80 and 120 mgm. per 100 cc. of 0.7 per cent sodium chloride solution. The results of these observa-

tions are given in table 9. A considerable decrease in the red corpuscle count, which can hardly be explained in any other way than as a direct haemolytic effect of the splenic extract, was obtained. Also the effect was progressive so far as these observations go.

A number of investigators have sought for direct haemolytic action of splenic extracts outside of the body. Nolf (4), Weil (5), Banti (6) and Furno (7) obtained positive results, but Achard, Foix and Salin (8), Widai, Abrami and Brulé (9) and Krumbhaar and Musser (10) were unable to detect any direct haemolytic action of fresh extract of spleen. In these observations the splenic extract was allowed to act upon washed red corpuscles rather than whole, freshly drawn blood. It is probable that washing the corpuscles removes the weaker ones and retains only the more resistant, which the splenic substance is unable to destroy. If the haemolytic substance in the extract of spleen is capable of attack-

TABLE 9

*Decrease in erythrocyte count produced by mixing blood with splenic extract outside of the body*

NUMBER OF OBSERVATIONS	BLOOD DILUTED WITH NORMAL SALINE	BLOOD DILUTED WITH NORMAL SALINE PLUS SPLENIC EXTRACT	AMOUNT OF SPLENIC EXTRACT PER 100 CC. OF DILUTING FLUID	AMOUNT OF DECREASE	PERCENTAGE DECREASE
			mgm.		
14	6,229,800	5,515,800	20	714,000	11.46
10	6,689,600	5,832,000	40	857,600	12.81
10	6,248,000	5,139,200	80	1,108,800	17.74
6	7,008,000	4,771,000	120	2,237,000	31.92

ing the less resistant corpuscles only we can understand how it was that in the experiments just quoted no reduction of reds was observed, whereas, in our experiments, in which fresh whole blood was used, a marked diminution in their number took place.

Zelenski (11) in 1891 and Danilewski (12) in 1895 were able to cause a marked increase in the number of red corpuscles in the circulating blood and also in the haemoglobin content of the blood following a single intraperitoneal injection of splenic extract. Paton, Gulland and Fowler (13) repeated their experiments without being able to detect any change in the erythrocytes. Silvestri (14) believed that he obtained an increase in the red corpuscle content of the blood in anaemia by the use of injections of splenic extract. Krumbhaar and Musser (10) also were able to bring about an increase in the erythrocyte count and haemoglobin content by the injection of fresh splenic extract. This



increase lasted one or two days and could be re-obtained by repeating the dose.

In each of these cases a blood count was not made until the day following the injection, whereas our counts were begun immediately after the injection and continued at short intervals for several hours. It was at this time the decrease noted by us occurred (see tables 1, 2, 4, 5, 7 and 8 for duration of effect). However, we have noted that when an animal was used for a second experiment after the lapse of a few days its initial red corpuscle count was frequently higher than it had been before any of the extract was administered. The initial count in experiment 21 is 7,216,000. A few days later this rabbit was used again, experiment 30, and then its initial red corpuscle count was 9,632,000; nevertheless a considerable decrease in the erythrocyte count (38.87 per cent) was promptly brought about by the injection of splenic extract.

The bone marrow usually responds to a lowering by hemorrhage and other means of the number of corpuscles in the circulating blood by increased production of new corpuscles, so that in the present instance, where the immediate effect of the splenic agent is a decreased erythrocyte count, it is reasonable to expect subsequently increased production. This compensation might very possibly exceed the loss especially if, as has been claimed by Stradomsky (15), the product of splenic activity, in addition to acting directly to destroy red corpuscles, exerts a stimulating influence on the bone marrow. The results obtained in the group of experiments in which 40 mgm. of splenic extract per kilogram of body weight were injected subcutaneously (table 8) are in accord with this idea of compensatory action on the part of the bone marrow. The table shows that in this series of experiments the percentage decrease and the duration of effect were both less than when the dose was only 20 mgm. per kilogram (table 7). It is possible that the greater the amount of haemolytic agent present the more prompt and more marked is the compensating response. It is more probable that Stradomsky's idea is correct and that the stimulating element of the splenic extract becomes predominant in the larger doses where it is undoubtedly combined with the independent effort of the bone marrow to replace the corpuscles that are being rapidly destroyed.

It is also possible that the effect described may be due to a depression of the haematopoietic activity of the bone marrow by the splenic extract, or to a temporary withdrawal of red corpuscles from the circulation. Such assumptions, however, are unsupported by any evidence of which we are aware.

We believe our results indicate the existence in the spleen of two substances exerting an influence on the number of red corpuscles in the circulating blood—one, a direct haemolytic agent, acting particularly upon the older and less resistant corpuscles; the other, an agent stimulating the production of new corpuscles by the bone marrow to replace the ones destroyed; both operating to keep the mass of corpuscles in circulation of the most efficient quality. Further research in this connection is being undertaken.

#### CONCLUSIONS

1. The subcutaneous injection of protein-free splenic extract is followed immediately by a decrease in the number of erythrocytes in the circulating blood.
2. The decrease is temporary.
3. The decrease is probably due to a direct haemolytic action of the splenic agent.
4. The decrease is frequently accompanied by a very transient increase in the number of white corpuscles.

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## PHYSIOLOGIC CHANGES PRODUCED BY VARIATIONS IN LUNG DISTENTION

### II. EFFICIENCY OF THE PULMONARY CIRCULATION IN OVER- COMING OBSTRUCTION

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The experiments on dogs, the results of which are recorded below, are part of a series undertaken to study the relationship between the pulmonary and the systemic blood pressures during distention of the lungs. The endeavor was to distend the lungs while eliminating as many as possible other respiratory factors which might influence blood pressure. To maintain distention of the lungs the entire animal was placed in a plethysmograph from which air could be exhausted and through the wall of which passed a tube allowing tracheal communication with the outside air. Similar tubes permitted exterior connection to recording manometers (1).

With distention of the lungs brought about by this method there was found to exist a surprisingly constant effect upon the carotid blood pressure which could be predicted for the degree of exhaustion of the plethysmograph. With very slight distention of the lungs, there occurred a small rise in blood pressure; with greater distention a marked fall, and with sufficient distention the blood pressure fell to within a few millimeters of the zero of the manometer and carotid pulsations ceased. These phenomena, in view of the fact that the heart continued beating vigorously during their occurrence, indicate a mechanical blocking of the circulation in the blood vessels of the lungs; that this blocking is due to occlusion of the pulmonary capillaries is further evidenced by a rise in pulmonary arterial pressure. In the more extreme degrees the lumen of the pulmonary capillaries was sufficiently reduced to prevent the passage of blood causing a rise in pressure in the pulmonary artery sufficient to distend the right heart.

The degree of negative pressure necessary to obliterate the lumen of the pulmonary vessels may be taken as a measure of the pulmonary arterial blood pressure. To use this method for an actual determination of the pulmonary blood pressure it is necessary merely to determine the minimal negative plethysmographic pressure that will cause disappearance of the pulse in the carotid artery. This degree of pressure can also be estimated by comparing the plethysmographic pressure with the blood pressure and determining the point where the fluctuation in one is equal to the fluctuation in the other. Both conditions indicate zero carotid pressure resulting from obstructed pulmonary capillaries. A more accurate method is to record the intrathoracic pressure for comparison with the negative plethysmographic pressure. In view of the fact that, by this method, the egress of blood from the pulmonary arterial circuit is prevented, the estimate of pulmonary arterial pressure indicates the maximum pressure attainable in this system, and our figures in consequence are considerably above the average usually given for pulmonary arterial blood pressure. They really indicate the maximum efficiency of the right ventricle to overcome resistance. The rise in pulmonary blood pressure will be discussed in a subsequent paper.

*Results of experimentation.* Since blood pressures and plethysmographic pressures were recorded by mercury manometers, the readings in millimeters of mercury on the unreduced tracings have been multiplied by 2 for correct determinations of pressure changes.

Experimental results are reproduced in figure 1; the sections of the tracing being characteristic parts from a series of thirty-one tests made on a single animal. It is noted that a fall in plethysmographic pressure produced a synchronous fall in blood pressure. This drop in blood pressure was always greater than the corresponding decrease in plethysmographic pressure. Any variation in plethysmographic pressure produced a corresponding change in carotid pressure. When the plethysmographic pressure returns to the atmospheric level, the carotid blood pressure rises to a level higher than that which obtained prior to the experiment; this rise is only transient, however.

The last three sections of the tracing show that there is a decrease in pulse pressure during the distention of the lungs and that there is also an absence of all respiratory waves in the blood pressure curve due to reflex apnoea. A comparison of the pulse pressure in *C* previous to the experimental distention of the lungs with that in *F* shows that there is an increase notwithstanding the fact that the animal had been

given considerable time in which to recover under normal atmospheric conditions from the effects of the previous experiments. This increase in pulse pressure after the performance of several experiments upon an animal is a characteristic to be noted on a large proportion of all tracings as is also the accompanying slow pulse rate. Two tracings are shown in *F* at nearly the same plethysmographic pressures; in the first of these the plethysmographic pressure was held constant after the maximum reduction in pressure, then suddenly released. In the second, the plethysmographic pressure was varied and the carotid pressure shows corresponding variations even at the low pressure level

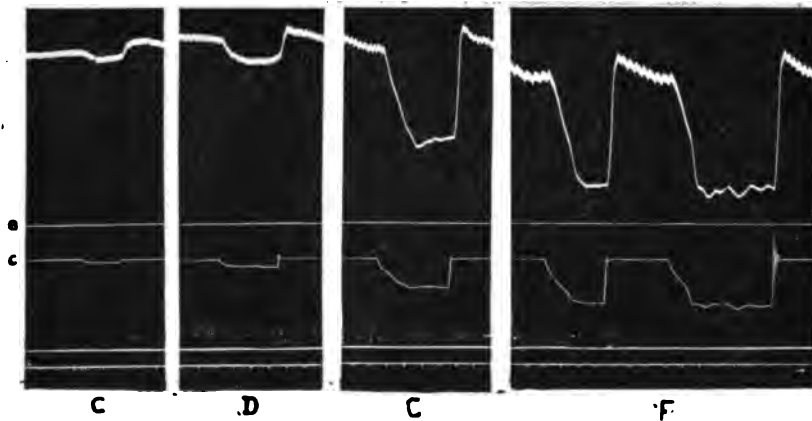


Fig. 1. Experiment of December 4, 1916. Dog of 7½ kilo. Ether. Time every ten seconds. A, Carotid blood pressure recorded by mercury manometer. B, Abscissa for carotid blood pressure which is 62 mm. above the time record. C, Pressure in plethysmograph recorded by mercury manometer.

at which these variations occurred. It will be noted that in each instance the variations in carotid pressure are greater than the corresponding variations in plethysmographic pressure. So consistently has this occurred with all negative pressures insufficient to completely occlude the pulmonary capillaries, that a fall in carotid pressure equal to the fall in plethysmographic pressure has been taken as an indication of pulmonary occlusion resulting in a depleted systemic arterial circulation and consequent zero arterial pressure.

The results of another procedure are shown in figure 2. In this experiment the pressure in the plethysmograph was gradually decreased to -94 mm. of mercury and subsequently slowly returned to the

atmospheric pressure. Here again it is noted that the fall in carotid pressure is not at all in proportion to the negative pressure in the plethysmograph until the point marked with the arrow is reached. It is at this point that the heart beats failed to record on the carotid pressure tracing, although the heart was vigorously beating at this time. Beyond this point to the point of release of plethysmographic pressure the two curves maintain practically an equal distance by vertical measurement between them, there being a difference of only 2 mm. The maintenance of this constant distance between the two curves, vertically measured, indicates that during this part of the experiment

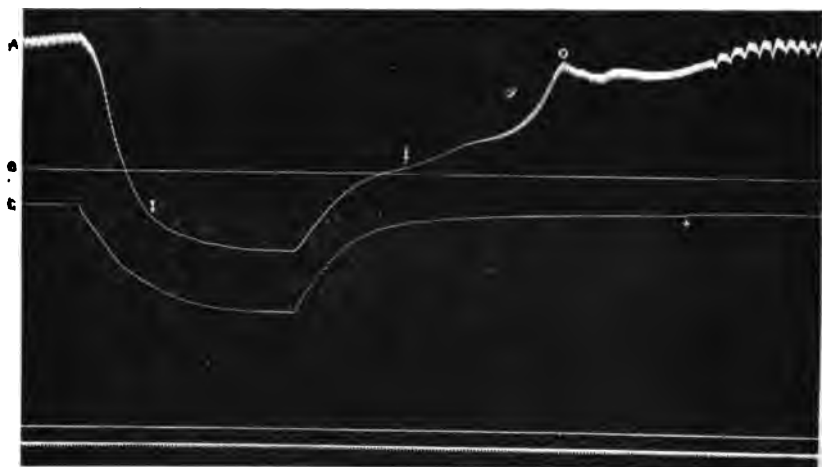


Fig. 2. Experiment of December 1, 1916. Dog about 7.5 kilo. Ether. Time every second. A, Carotid blood pressure recorded by mercury manometer. B, Abscissa for carotid blood pressure which is 121 mm. above the time record. C, Pressure in plethysmograph recorded by mercury manometer. Arrows indicate points of disappearance and reappearance of pulse waves in unreduced tracing. Cross indicates point at which the plethysmographic pressure was finally released. Circle indicates an oscillation common to many tracings.

the fall in carotid pressure is due exclusively to the fall in plethysmographic pressure and not to changes in the animal's blood-pressure mechanism. In other words, the pulmonary circulation is completely blocked and there is a consequent complete failure in the systemic circulation. The degree of negative pressure necessary in this experiment to occlude the pulmonary vessels measured on the original tracing (from the point where the two curves commence to maintain an equal

vertical distance between them) was found to be 40 mm. which, multiplied by 2, equals approximately the maximum pulmonary arterial pressure obtainable in this animal under the favorable conditions of occluded peripheral pulmonary vessels.

On the gradual release of the plethysmographic pressure, reestablishment of the systemic circulation takes place, and the heart beats again show at the point indicated by the second arrow. It is at this point that the rise in carotid blood pressure becomes greater than the corresponding rise in plethysmographic pressure. The systemic circulation does not become reestablished until a level of plethysmographic pressure considerably higher than that necessary to deplete it has been obtained. The probable explanation of this lies in the fact that the cardiac muscle has suffered during the period of depleted systemic circulation of which the coronary arteries are a part. The high pulmonary arterial pressure and consequent high intraventricular pressure on the right side of the heart which under these conditions persists even during the ventricular diastole is an added factor preventing the transmission of blood through the right heart muscle itself. A systemic diastolic pressure higher than the right ventricular diastolic pressure is necessary for the circulation of the blood through the muscle of the right heart. The time factor is therefore of importance and to eliminate cardiac failure as a cause in the fall in carotid pressure the duration of an experiment must be short. When the blood pressure approaches its pre-experimental level a variation is seen (circle) which might well be due to the fact that the left heart is no longer flooded with the excess blood which had been retained in the pulmonary arteries by the capillary obstruction due to distention of the lungs. A reflex apnoea is evidenced by the absence of respiratory waves until the plethysmographic pressure again reaches that of the atmospheric pressure at the point marked +.

The results of 49 typical experiments upon five dogs are given in table 1 and the curves plotted from these data shown in figure 3. The graph is so drawn as to show the fall in carotid pressure (ordinates) caused by given changes in plethysmographic pressures (abscissae) which range from zero to a negative pressure of 118 mm. of mercury. The dots indicate plethysmographic pressures and the circles the fall in carotid blood pressure resulting from these negative pressures.

In plotting the fall in carotid pressure due to a given degree of negative plethysmographic pressure, the fact has been taken into consideration that, had the condition of the animal's circulation remained

TABLE 1

	CAROTID PRESSURE BEFORE EXPERIMENT	PLETHYSMO- GRAPHIC PRESSURE	CAROTID PRESSURE DURING EXPERIMENT	FALL IN CAROTID PRESSURE CORRECTED	CAROTID PRESSURE AFTER EXPERIMENT	DURATION OF EXPERIMENT IN SECONDS
Dog I.....	113	-1	113	+1	113	56
	113	-2	113	+2	113	20
	121	-4	121	+4	135	30
	143	-5	133	5	123	38
	131	-9	116	6	139	35
	121	-10	90	.21	120	30
	148	-12	128	9	167	30
	135	-16	94	25	140	36
	146	-20	90	36	145	45
	138	-25	75	38	146	42
	141	-31	54	56	128	25
	150	-46	19	85	146	31
	148	-50	-16	114	104	35
	133	-60	-46	119	90	38
	134	-70	-57	121	83	38
	152	-3	146	3	161	20
	155	-6	143	6	168	28
Dog II.....	162	-8	142	12	169	32
	146	-13	116	17	80	40
	142	-14	101	27	166	40
	147	-18	108	21	175	50
	144	-19	90	34	171	48
	150	-22	100	29	174	70
	153	-26	73	54	171	40
	149	-33	56	60	171	42
	134	-36	45	53	150	48
	158	-37	48	73	159	40
	146	-40	38	65	156	40
	136	-41	35	61	147	50
	128	-47	17	64	147	37
	138	-15	101	23	138	28
Dog III.....	130	-17	83	30	113	31
	115	-28	42	45	108	32
	138	-38	25	75	129	35
	104	-39	2	63	120	30
	119	-59	-33	93	140	38
	143	-72	-32	103	156	32
	129	-73	-50	106	143	35
	140	-77	-55	118	145	45
	145	-85	-60	120	119	50
	119	-95	-72	96	116	52



TABLE 1—*Concluded*

	CAROTID PRESSURE BEFORE EXPERIMENT	PLETHYSMO- GRAPHIC PRESSURE	CAROTID PRESSURE DURING EXPERIMENT	FALL IN CAROTID PRESSURE CORRECTED	CAROTID PRESSURE AFTER EXPERIMENT	DURATION OF EXPERIMENT IN SECONDS
Dog IV.....	116	-23	35	52	118	47
	129	-45	-7	91	125	29
	125	-117	-93	101	117	60
	118	-118	-74	74	102	53
Dog V.....	157	-34	42	81	174	27
	163	-52	8	103	164	11
	166	-90	-68	144	150	18
	150	-102	-88	136	150	18

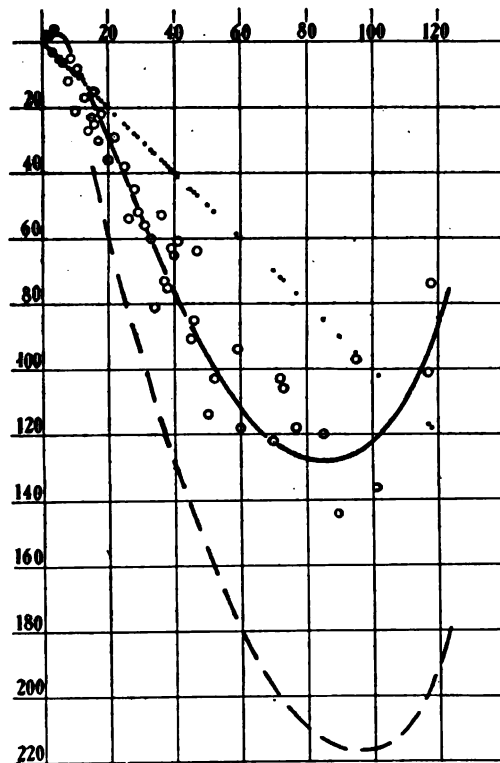


Fig. 3. Composite plotted curves (5 dogs) showing typical behavior of carotid pressure during forty-nine observations at varying degrees of negative plethysmographic pressure. Circles indicate corrected fall in blood pressure. Dots indicate plethysmographic pressure. Ordinates indicate fall in carotid pressures. Abscissae indicate negative plethysmographic pressures. Broken line represents average curve of uncorrected fall in carotid pressure. Unbroken line represents average curve of corrected fall in carotid pressure.

unchanged, the fall in carotid pressure would have equalled the fall in plethysmographic pressure, since both manometers were affected by the same variations within the plethysmograph. The fall in carotid pressure in excess of that in the plethysmograph shows that circulatory changes also occurred within the animal. The difference between the plethysmographic and carotid falls, therefore, indicates the extent of these changes, and it is this difference representing the changes brought about in the animal's circulatory mechanism which has been plotted as the corrected fall in carotid blood pressure.

From these data we have plotted an average curve (unbroken line) for all negative pressures between zero and 118 mm. of mercury. An additional average curve (broken line) of fall in carotid blood pressure has also been drawn to show the fall without the correction made in the first curve.

Both of the average curves serve as a measure of the maximum pulmonary pressure obtainable under the conditions of distended lungs and blocked pulmonary peripheral vessels. It has been shown in previous figures that the carotid blood pressure undergoes a greater variation than does the plethysmographic pressure, provided that the plethysmographic negative pressure is not sufficient to cause a complete blocking of the pulmonary capillaries as a result of which the carotid blood pressure equals zero. The minimal negative pressure which causes a complete blocking in the pulmonary vessels is estimated at the point where the carotid curve commences to show variations equal to instead of in excess of that of the plethysmograph. In the uncorrected (broken line) curve of carotid blood pressure the point at which this curve is parallel to that of the plethysmographic pressure is the index of the maximum pulmonary blood pressure. The degree of negative pressure in the plethysmograph necessary to produce this parallelism is 85 mm. of mercury. In the corrected curve (unbroken line) the index is the degree of negative plethysmographic pressure necessary to produce parallelism between the carotid curve and an abscissa line, since the plethysmographic negative pressure was subtracted from the fall in carotid blood pressure before drawing the curve. It will be noted that the plethysmographic pressure necessary to produce the latter parallelism is the same as in the corrected curve, 85 mm. of mercury.

A further analysis of the corrected fall in carotid blood pressure shows that with very slight negative plethysmographic pressures (zero to 5 mm. of mercury) the average carotid pressure shows a small rise. The

actual fall in intrathoracic pressure is assumed to be less than the fall in plethysmographic pressure, because the latter must overcome the resistance offered by the elasticity of the lungs before it can affect the former. It is for this reason that 5 mm. of mercury is referred to as a slight negative plethysmographic pressure. With a fall of from 5 to 10 mm., on the other hand, the blood pressure shows an actual fall but not to the same extent as the plethysmographic pressure. With still greater negative plethysmographic pressures, 10 mm. to 118 mm., the average blood pressure curve falls to a much greater extent than the plethysmographic, the maximum fall of 128 mm. being attained with a plethysmographic pressure fall of 85 mm. Variations in the fall of carotid pressure are probably due to differences in lung elasticity in different animals and to differences in initial blood pressure.

When the negative plethysmographic pressures are increased below 85 mm., the fall in blood pressure grows proportionately less and finally the blood pressure begins to rise. Emphysema and pneumothorax which were produced at these low pressures offer the most probable explanation of this phenomenon.

#### GENERAL CONSIDERATIONS

Increase in the capacity of the pulmonary blood vessels brought about by the normal act of inspiration, causes accumulation of blood in the lungs and a corresponding fall in carotid pressure due to the transient retardation of blood flow to the left heart (2). However if at the end of inspiration no further change is made in the thoracic capacity, no further accumulation can occur and a constant stream should again return to the left auricle with a consequent return of blood pressure to its normal level. It therefore is certain that a fall in carotid pressure, such as has been shown to occur in our experiments and lasting as it does through a long period during which the lungs remain evenly distended, cannot be explained as a result of accumulation of blood in the pulmonary vascular area. The amount of blood locked up in the pulmonary vessels under the condition of prolonged distention of the lungs is so small that it may well be neglected as a factor in determining the fall in general blood pressure under our method of experimentation.

The changes in abdominal pressure brought about by distention of the lungs by the method adopted by us, at first would seem to be the opposite of those brought about by natural respiration. One can readily see the abdomen distend under negative pressure and this

creates the impression that the abdominal walls are being acted on by a force pulling them outward and causing a negative pressure in the abdomen which if it occurred would cause engorgement of the visceral vessels and retard return flow of blood to the heart. But, when all the conditions of pressure on the animal are considered, it is seen that the only change in relative pressures that has occurred is in the animal's relation to its intrapulmonic pressure. In other words, there is a pressure in the alveoli of the lungs, greater than the pressure to which the rest of the animal is exposed. The consequent descent of the diaphragm increases relatively the abdominal pressure, as it does in natural respiration. Post-mortem examinations of the abdominal viscera of animals allowed to die under low negative pressure have clearly shown that, far from having an accumulation of blood in these viscera such as would be the case if the abdominal pressure were reduced, there is on the contrary a marked diminution in the amount of blood present in these organs, evidenced by a very distinct blanching. Expansion of gases in the alimentary tract possibly favors this blanching. The fall in carotid blood pressure cannot therefore be explained by the assumption of a bleeding into the abdominal vessels under low pressure.

When artificial respiration was maintained by rhythmically varying the negative pressure in the plethysmograph the muscles remain in apnoeic passivity so that variations in blood flow through them are negligible.

It is conceivable that changes might occur in the position of the crura of the diaphragm which would offer an obstruction to the return of blood to the right heart and thus cause a fall in general arterial blood pressure. Such a condition evidently would tend to congestion of the abdominal viscera, which we have found to be absent. There is furthermore a coincident rise in pulmonary blood pressure, a fact which is not in conformity with obstruction to the entrance of blood into the thorax. We may then eliminate such crural obstruction as a causative factor in the fall of carotid blood pressure.

In many of our experiments the fall in blood pressure occurred without change in heart rate and since apnoea was often caused by the distention of the lungs, the nervous factor of overflow from the respiratory center to the vagus center inhibiting the latter and causing a more rapid heart beat, need not be considered as of influence on the resultant blood pressure. Further indications that change in heart rate brought about by nervous control is not responsible for the fall in blood pressure are the facts that the fall occurs with both vagi cut or

frozen. Changes in heart volume will be discussed in subsequent paragraphs with reference to the supply of blood to the left heart.

The fall in systemic blood pressure is not due to vasodilatation. The initial fall is too rapid to be thus accounted for. Cerebral anemia resulting from prolonged periods of lung distention causes, on the contrary, vasoconstriction; revealed by a gradual and continued rise in the pressure level in the carotid artery and by the blanching of the viscera already alluded to.

The elimination of the above factors as mediating the fall in blood pressure, coupled with positive findings such as a rise in pressure in the pulmonary artery and distention of the right heart indicate clearly that we are dealing simply with an obstruction to the flow of blood through the lung capillaries. The mechanics of this obstruction require further consideration.

Cloetta (3) has expressed the view that

as the lungs expand two opposing factors act upon the intrapulmonary vessels. When the alveoli first expand, they tend to exert a radical traction upon the small vessels and capillaries and so enlarge them, but as the enlargement proceeds and the alveoli acquire a polygonal shape, they tend to compress the intrapulmonic vessels. Furthermore, as the lungs enlarge, they necessarily cause a linear extension of the blood vessels and thereby further reduce their calibre. From this it appears that a moderate distention of the lungs causes a diminished resistance, but an extreme distention an augmented resistance.

In the case of Cloetta's method of lung distention in which the lungs alone are contained in the plethysmograph, the heart and large blood vessels are not subjected to variations in pressure synchronously with the lungs as occurs in normal respiration, and as de Jager (4) has pointed out, the capacity of the large extra pulmonary vessels may increase when the surrounding pressure becomes more negative. It is noteworthy that by subjecting the entire animal to negative pressure as we have done, in contrast to the method of Cloetta, the aspiratory effect due to enlargement of the blood vessels by distention of the thoracic cavity is negligible, if we disregard the fact that the thoracic and abdominal cavities are more expansible than the solid tissues of the body.

Concerning the rise in carotid blood pressure with very moderate negative pressure in the plethysmograph and the fall with greater pressures, our findings with a somewhat different method are in conformity with those of Cloetta. But his explanation of these phenomena based on the hypothesis of polygonally shaped alveoli making

pressure on the pulmonary blood vessels, is open to objections which seem of sufficient weight to exclude it. Even if the blood vessels of the lungs could be as nicely placed in reference to the surrounding cells, as assumed by Cloetta (fig. 4) a fractional part of the elastic force of the distended air sacs might be exerted on the walls of the blood vessels only by the assumption of a shape and deformity such as is

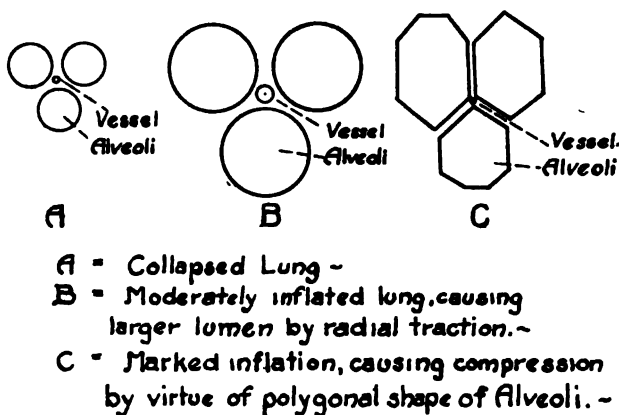


Fig. 4. Diagrams illustrating the effect of lung inflation on the alveoli and lumen of the intrapulmonary vessels (after Cloetta).

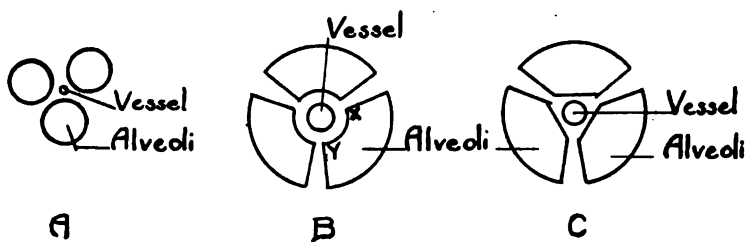


Fig. 5. Diagrams illustrating effect on the caliber of the pulmonary blood vessels produced by change in shape of the alveoli.

illustrated in figure 5, B. Should the walls of the alveoli be sufficiently movable for the elastic pull due to their distention to be equally exerted at all points in their circumference in directions tangent to the surfaces at those points, it would follow that the effect of this tangential pull would be to straighten the line *xy* (fig. 5) thereby compressing the blood vessel; but such a resultant pressure could be obtained only when a

part of the curvature of the alveolus was concave with reference to the blood vessel, and never with polygonally shaped cells in which the resultant of the elastic pull inward for converse reasons would be accentuated at the angles.

While stretching of the capillaries of the lungs in their long diameter causing a diminution in their caliber and obstruction to blood flow is undoubtedly a factor in the fall in carotid pressure, it does not seem probable that it can result in complete occlusion of the vessels. The extent to which capillary diminution can be brought about by this means is under investigation.

Of the factors that might be the cause of the fall in carotid blood pressure, there remains the relative increase in intrapulmonic air pressure, which apparently is the essential cause.

In considering the pressure of the intrapulmonic air in its effect on the pulmonary vessels, under the conditions of our experiments, it must be borne in mind that while there is no change in the relation of the intrapulmonic air to the atmospheric air while the lungs are distended, yet there is a considerable change in the intrapulmonic air with reference to the animal subjected to the negative pressure. If an animal be subjected to a given negative pressure while his intrapulmonic air is still in free communication with air at atmospheric pressure it is evident that distention of the air sacs commences and continues until this elasticity plus the elastic resistance offered to increase in the capacity of the thorax equals the increased intrapulmonic pressure. The factor responsible for the distention of the lungs is the difference between the intrapulmonic pressure and the external pressure on the animal; it would be indifferent whether this distending pressure is obtained by diminishing the external pressure on the animal or by increasing the intrapulmonic pressure. It is furthermore clear that under either of the conditions stated above, vessels situated between the alveoli, as the pulmonary capillaries are, must likewise be subjected to the distended pressure. In other words, the pulmonary capillaries lying between adjacent alveoli are compressed between two opposing forces when the intrapulmonic air pressure is increased.

It would follow from this that with varying degrees of negative pressure in the plethysmograph corresponding degrees of pressure exerted in the pulmonary vessels impede proportionately the pulmonary circulation. The fall in carotid pressure is due solely to this interference with the return of blood to the left heart. The total failure of systemic circulation is due to the fact that even the maximal pressure

obtainable in the pulmonary arteries is not sufficient to overcome an external resistance of more than 85 mm. of mercury. This maximal pressure, attained during the period of complete occlusion of the pulmonary vessels, while indicating relative inefficiency in the muscles of the right ventricle compared to those of the left side of the heart, yet is considerably greater than is usually given for average pulmonary arterial blood pressure.

We are indebted to Prof. W. E. Garrey for helpful suggestions and criticisms.

#### CONCLUSIONS

1. When the lungs are artificially distended by diminishing plethysmographic pressure: *a*, with slight distention there is an inconspicuous rise in carotid blood pressure due to the freer passage of blood to the left heart; *b*, a moderate distention always causes a fall in carotid pressure, the greater the negative pressure in the plethysmograph the greater is the reduction of the carotid pressure, the fall in carotid pressure always being greater than the causative negative pressure in the plethysmograph; *c*, with full distention there occurs a fall in carotid pressure approximately to zero; *d*, with over-distention, causing emphysema and pneumothorax, there is a slighter fall than that produced by less distention.

2. The fall in carotid blood pressure caused by moderately diminished plethysmographic pressure is due to failure in the supply of blood to the left heart.

3. This failure of blood supply to the left heart is due to pressure exerted almost directly upon the small pulmonary vessels.

4. The measure of the degrees of pressure necessary to occlude the vessels can be taken as an index of the pressure in the pulmonary arteries.

5. During a short period of occlusion of the pulmonary capillaries, the blood in the pulmonary arteries may attain a pressure of 85 mm. of mercury.

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## EXPERIMENTS ON THE PATHOLOGICAL PHYSIOLOGY OF ACUTE PHOSGENE POISONING

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Phosgene is one of the lethal asphyxiant war gases. If its effects on the animal organism were to be described in one phase, it would be by the words "pulmonary edema." A study of phosgene poisoning is therefore of more than passing medical interest. The work here reported was done by the Wisconsin Section of the Medical Division of the Chemical Warfare Service in an attempt to get a physiological background for proposed forms of treatment.

The general symptoms of gas poisoning have recently been well described by Underhill (1) and since our experience on this point is identical with his, such description need not be repeated here. Underhill's division of phosgene poisoning into three stages is also in accord with our findings, and although arbitrary in certain cases, as all such classifications must be, it is of distinct advantage in locating the various physiological disturbances and in gaining an insight into the condition as a whole.

Our work has consisted in following as closely as possible various physiological reactions throughout the course of fatal phosgene poisonings. We have directed particular attention to the following: arterial blood pressure, venous blood pressure, pulse rate, hemoglobin

<sup>1</sup> The work here reported was done in the Medical School Laboratories of the University of Wisconsin. Problems of gas warfare were initiated under the Bureau of Mines and continued under the Wisconsin Section, Medical Division, Chemical Warfare Service. In addition to the authors, who directed and carried on the major part of this research, especial mention should be made of the services of Dr. S. A. Amberg of the Otho S. A. Sprague Institute, and Mr. J. Y. Malone of the Wisconsin Section, who greatly aided in many phases of the work. Among others associated in a general way with the physiological studies carried on at Wisconsin, there should be mentioned Dr. H. S. Gasser of the Washington University Medical School, and Dr. B. H. Schlomovitz, Dr. P. F. Clark and Mr. L. F. Richdorf of the Wisconsin Section.

determinations, blood volume, red blood cell counts, histological examination of the lungs, x-ray studies of heart and lungs, alkaline reserve, respiratory rate and volume of respiratory ventilation.

*Methods.* Dogs were used throughout these studies. The animals were subjected for thirty minutes to air containing 80 to 100 parts per million of phosgene. This was sufficient, with rare exceptions, to produce death in the first twenty-four hours. The technique of this gas administration was one gradually evolved at the various Chemical Warfare laboratories. It consisted of placing the dogs in a one-hundred-liter air-tight glass box through which air was drawn at the rate of one hundred liters per minute. The phosgene cylinder was connected to the air inlet tube, the gas outflow being regulated by a needle valve and the rate roughly determined by a flowmeter. From the gassing chamber itself air was drawn in order to determine by chemical analysis the exact concentration of phosgene to which the animals were subjected.

All animals on which it was necessary to make incisions were morphinized either before or immediately after being gassed. A series of morphine controls had been carefully studied and we are sure none of the effects to be described are attributable to morphine. In order to make observations rapidly and frequently the animals were kept tied to operating boards. Since no pressure was exerted anywhere except by the cords on the limbs, and the respiratory passages were entirely unobstructed, this restraint seemed unobjectionable. The animals lay quietly and comfortably until the usual asphyxial stimulations occurred shortly before death.

*Arterial blood pressure.* By attaching a mercury manometer to the femoral artery, arterial blood pressure records were made in the usual way at half-hour intervals. The general course of the blood pressure in a typical case of phosgene poisoning may be seen at a glance by referring to figure 1. This composite curve, in common with those that are to follow, was made by dividing each of the experiments in the series into ten equal periods. Our animals lived an average of sixteen hours after gassing. Each of the ten intervals therefore represents on the average a little over one and a half hour. The data for the same period in all the experiments were averaged and the results plotted as a composite curve.

A few animals showed a slight fall of blood pressure after being taken from the gassing chamber. In most cases this was insignificant and it did not lower the composite curve during the first period. As

a rule the blood pressure gradually rose during the first half of the experiment increasing some 10 per cent above the normal. Beginning with the sixth period it began to fall slowly, reaching normal at the beginning of the eighth interval. Once having passed the normal the decline became extremely rapid and continued without intermission until the death of the animal.

This break in blood pressure which occurred at the eighth period was an extremely striking event in all the animals studied. It made

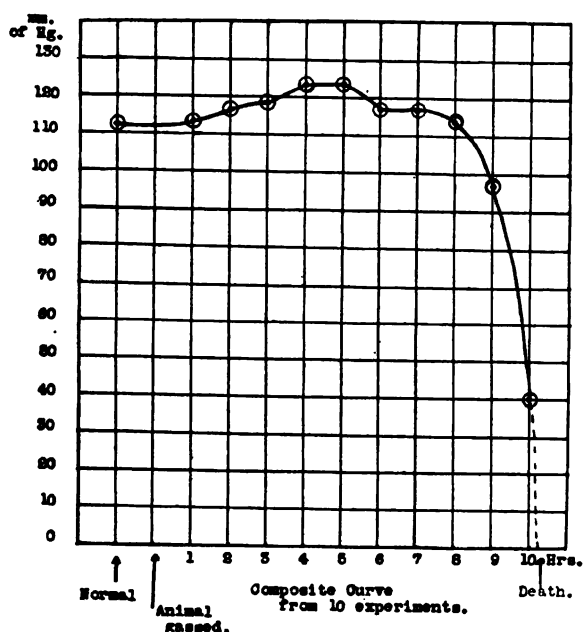


Fig. 1. A composite curve of arterial blood pressure in acute phosgene poisoning. The abscissa indicates the ten periods into which each experiment has been divided.

possible a very accurate prediction as to how much longer the animal would survive. Until its significance was fully appreciated, many of the animals died before final observations could be made.

Figure 2 is a reproduction of the actual records from a typical experiment. The points just mentioned may be noted. The first record at 11:10 a.m. was taken shortly after gassing. The pressure then rose gradually for more than six hours. At 12:10 a.m., thirteen hours after gassing, the pressure was still normal, though falling. One hour and twenty minutes later the animal was dead.

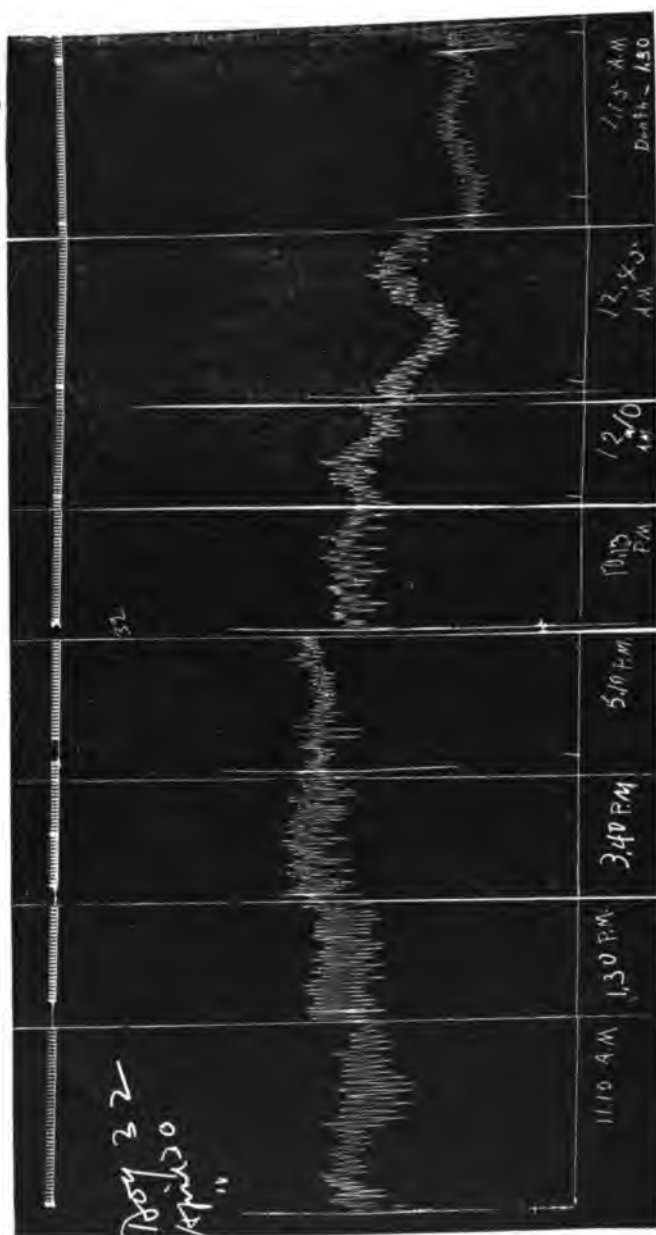


Fig. 2. Arterial blood pressure record from a case of acute phosgene poisoning.

One need not attempt to interpret the arterial blood pressure curve of acute phosgene poisoning until all the other data have been presented. Its resemblance to an asphyxial vasoconstriction is however obvious. That the rise may be due to vasoconstriction and the fall in part to paralysis of the vasomotor center is also suggested by the large vasomotor waves which so often occurred late in the blood pressure tracings (see fig. 2 at 12:45 a.m.).

*Venous blood pressure.* In the preceding series of ten experiments venous pressure observations were made simultaneously with the arterial. These were secured by inserting a sound into the femoral vein. The sound was connected to a manometer and a reservoir of Ringer's solution. The pressure in the system was raised above what the reading was likely to be. On removing a clip the blood pressure was balanced against that of the fluid in the manometer. The reading could be made before there was any tendency to clot and the fluid added to the blood stream at each observation was negligible.

Venous pressure was found to be rather variable, conforming on the whole however to what one might expect from the arterial. During the long period of increased arterial pressure, venous pressure was either normal or slightly below. In the terminal stages, however, it often rose markedly. In two of the ten experiments there was a noticeable increase in venous pressure immediately after gassing. These were very severe cases, death occurring within nine hours. It would seem probable that in these animals the initial injury to the lungs was so great that the pulmonary circulation was obstructed and venous pressure therefore forced to rise.

*Pulse rate.* Shortly after gassing the pulse rate fell in practically all cases. This occurred in animals morphinized before gassing as well as in those that received none of the drug. It was therefore an expression of the action of the poison itself. The decrease in rate brought the pulse from an average of 95 to 70 beats per minute. By the time the experiment was half over the heart rate had returned to normal and following this it very markedly accelerated. Figure 3 presents a composite curve from fourteen experiments in which the pulse rate was carefully followed. The final determination of the curve is the average of the highest rates obtained in the tenth period. As death became imminent the heart rate became irregular and the rate then, of course, decreased. Electrocardiograms taken at this time showed various kinds of blocks, dropped beats and extra systoles, features which characterize most records taken during death by asphyxiation.

The pulse rate offered an excellent means of following the condition of the animal. Two of the dogs in one of our series recovered. The pulse curves from these animals were of special interest. There was the initial fall and the subsequent rise, but the latter never exceeded 120 beats per minute. A large number of observations have confirmed our opinion that a fatal outcome is to be expected if the heart rate continues to rise above 125 or 130 beats.

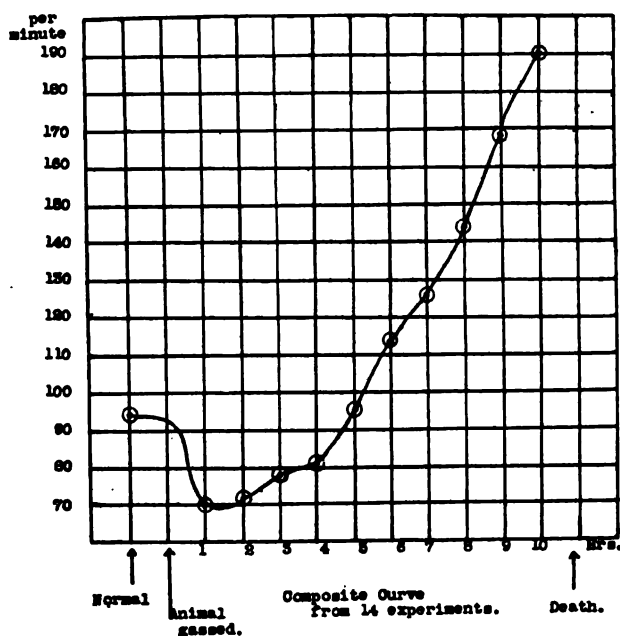


Fig. 3. A composite curve showing the average heart rate in acute phosgene poisoning.

*Hemoglobin determinations.* It was soon realized that there were very significant changes in the hemoglobin content of the blood in dogs suffering with phosgene poisoning. At death the blood was viscous, even tarry in consistency, and the hemoglobin readings showed an almost unbelievable concentration. Underhill first pointed out that this stage of concentration was preceded by an initial one in which the hemoglobin content of the blood was decreased.

The characteristic hemoglobin changes during the course of the poisoning may be seen in figure 4. In an average experiment the hemoglobin

readings were below normal during the first four periods of the experiment. This constitutes Underhill's first stage of phosgene poisoning. Concentration then began and in the period preceding death the hemoglobin readings averaged over 150 per cent. The periods of concentration make up Underhill's second stage.

The maximum decrease in hemoglobin occurred anywhere during the first four periods, in other words, during the first five or six hours of the experiment. Since the maximum decrease did not occur in the same period for all the experiments, the composite curve does not show the lowest limit reached in hemoglobin concentration. In the sixteen cases reported, it actually averaged 11 per cent, that is, a hemoglobin reading of 89. The lowest reading noted was 85, although in treatment

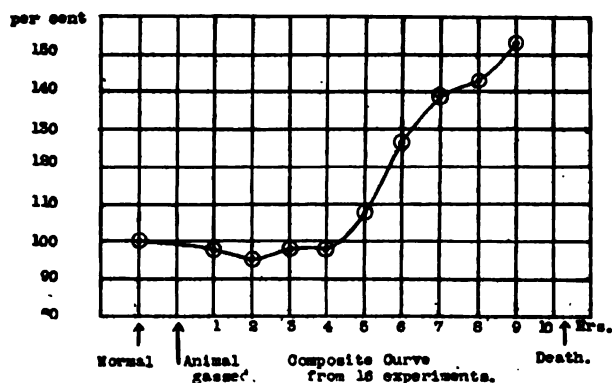


Fig. 4. A composite curve of hemoglobin concentration in acute phosgene poisoning.

series not here reported we have had readings as low as 80 and one of 78. The maximum decrease in our experience lasted a very brief time and often there was difficulty in making desired observations at exactly the proper moment.

The degree of subsequent hemoglobin concentration has always borne a definite relation to the severity of the poisoning and made possible a rather accurate prognosis. One hundred and twenty-five per cent may be said to represent a critical point. Any animal exceeding this figure was pretty sure to die in the course of the next five or six hours. If an animal was not gassed enough to reach this concentration, or if by any means a 125 per cent concentration could be prevented, there was an excellent chance for recovery.

Hemoglobin readings are usually interpreted in terms of the fluid content of the blood, or blood volume. If plasma or water has left the blood stream there is of course a concentration of hemoglobin, and if fluid has entered the blood stream from the tissues or elsewhere there is naturally a lowered hemoglobin content. While this is the general rule, there may be exceptions. Hemolysis or stagnation of red blood cells at any point might very greatly modify hemoglobin determinations and yet the blood volume would be entirely unchanged.

The natural interpretation of the hemoglobin curve for phosgene poisoning would be that in stage 1 there is an increase of blood volume and in stage 2 a marked decrease. That there is a real decrease in stage 2 is borne out by the fact that the lungs are now full of fluid which must, of course, have come from the blood. There is, however, no equally obvious explanation for an increase of blood volume in stage 1. Underhill (1) also finds in this stage a decrease in the blood chlorids, but the excess which he at the time finds in the urine and fluid of the lungs might account for this decrease.

In order if possible to clear the matter up we have made direct blood volume determinations during stages 1 and 2.

*Blood volume determinations.* The blood volume has been determined directly in eight animals during stage 1 and in three animals during stage 2. The technique used was the acacia method (2) which has been developed in this laboratory. In table 1 may be seen the results.

The data presented give no evidence of a blood volume increase in stage 1. In a large series of normal dogs the volume has frequently been 10 and 11 per cent of body weight with an average of 9.7 per cent. The eight animals here investigated averaged then within 2 per cent of normal. Furthermore of the three animals having the greatest hemoglobin dilutions, only one had a volume above the average.

In stage 2 only three determinations were made and unfortunately no hemoglobin readings were made simultaneously. Red blood cell counts, which have always paralleled the hemoglobin readings, indicated however that the latter were in the neighborhood of 135 per cent. Since the technique required the removal of 20 cc. of blood, the determinations were made some time before the anticipated death of the animal. The figures particularly the first and third show that these animals had a marked decrease in blood volume. Even 8.1 per cent body weight is a lower blood volume than we have ever found in a normal dog.



Stage 1 of phosgene poisoning, on the grounds of blood volume data, we believe to represent an actual decrease in the total hemoglobin content of the blood, a point which will be discussed later under the heading histological examination of the lungs.

In stage 2 there can be no doubt that the blood volume is actually greatly decreased.

*Red blood cell count.* Little need be said concerning the red blood cell counts other than that they uniformly paralleled the hemoglobin

TABLE 1  
*Showing blood volumes in phosgene poisoning*

WEIGHT OF ANIMALS	Hb. AT TIME OF DETERMINATION	BLOOD VOLUME IN PER CENT OF BODY WEIGHT
Stage 1		
<i>kgm.</i>	<i>per cent</i>	
9.80	88.4	8.7
9.58	90.9	9.6
9.14	91.0	11.1
9.82	94.4	10.8
8.48	96.0	8.7
8.42	92.0	9.5
7.22	83.5	11.2
5.70	84.5	9.9
Average.....		9.9
Stage 2		
14.00		6.5
13.8		8.1
13.5		7.5
Average.....		7.3

determinations. A composite curve from nine experiments may be seen in figure 5. As in the hemoglobin curve, stages 1 and 2 are evident and they occupy the same relative positions.

*Histological examination of the lungs.* Dr. W. S. Miller of the Department of Anatomy has kindly made a careful histological examination of lung tissue in a series of poisoned dogs. He has used his special methods for fixing and staining pulmonary tissues rather than the routine technique of general pathology. These examinations show that the injury from phosgene is almost exclusively in the lower

respiratory passages. There is constriction or spasm of the small bronchioles with the accompanying atelectasis and emphysema. There is edema of the connective tissue. The alveoli are irregular, their membranes injured, and in many cases they contain exudate. Very important from our point of view is the extensive clogging of the capillaries with red blood cells. Even small veins are solidly plugged. In many cases these masses in the veins have shrunk slightly and the surrounding clear areas are filled with serum. In figure 6 may be seen a photomicrograph illustrating the points just mentioned. That these changes in the lungs are not post-mortem is substantiated by the fact that they are characteristic of all the early

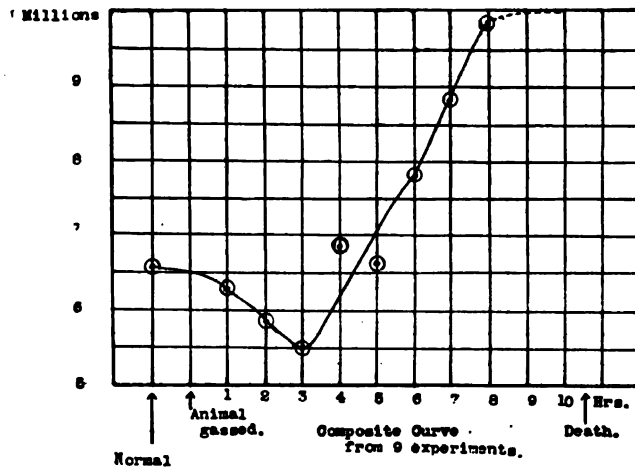


Fig. 5. Red blood cell counts in acute phosgene poisoning.

stages of poisoning and that they were found after every attempt to avoid post-mortem clot.

Furthermore, if phosgene in a dilution as great as 1 to 20,000, that is 0.222 milligrams per liter, be bubbled through a 2 per cent suspension of defibrinated dog's blood, there is in twenty minutes a marked agglomeration of the red corpuscles. This is direct evidence that the gas has the power of doing just what the histological picture shows has been done.

In slightly later stages than the one figured, there is evidence that compensatory paths are being opened up for the blood stream. Capillaries which were not plugged are widened and others have been dilated sufficiently to allow a flow of fluid around the obstructions.

The importance of these histological findings on the physiological conception of phosgene poisoning is at once apparent. The plugged capillaries and veins must at first greatly increase pulmonary resistance and the work of the right heart. Later there is relief by the develop-

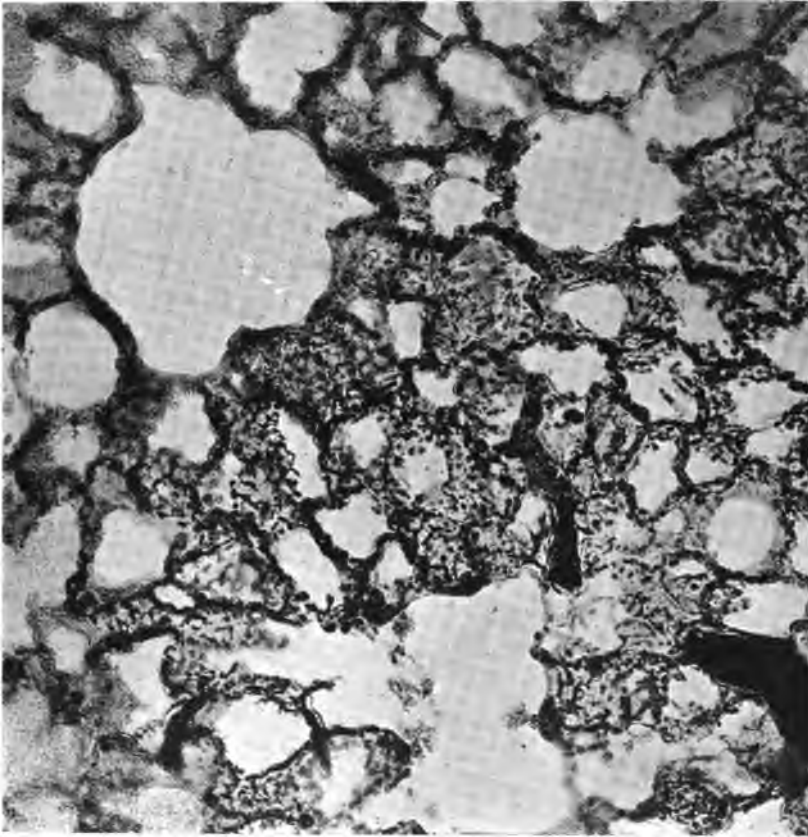


Fig. 6. A photomicrograph from the lower left lobe of a dog's lung, killed one hour and forty minutes after being gassed. Especially to be noted are the capillaries gorged with red blood cells, the alveolar exudate and the pulmonary vein in the lower right hand corner which is plugged with a mass of blood.

ment of compensatory passages. Just how the heart reacts to this we shall see in the following section. Furthermore the injuries to the alveolar walls must decrease the exchange of gases between the blood and the alveolar air.

*Heart size.* In a large series of experiments stereoscopic examinations and x-ray photographs of the thorax have been made at frequent intervals. At first the outline of the heart was sketched in with a grease pencil on the glass cover of the fluoroscope. Later stereoscopic plates were made and these, of course, proved much more reliable than the former method. To be sure that the animal was in the same position for each photograph, a lead cross was sewed to the chest and the center of this brought under a plumb bob at each exposure. Exposures were always made during the same phase of respiration, preferably inspiration, and the flashes were long enough to insure that it was the diastolic size of the heart which was secured.

These observations have shown two interesting and important changes in heart size during acute phosgene poisoning. There was first, immediately after removal from the gas chamber an increase in the size of the heart which varied considerably in degree, but was always associated with a relative enlargement of the right auricle and ventricle. This condition persisted for several hours and might even increase for an hour or more.

By the beginning of the fourth period, assuming that the experiment had been divided into ten equal intervals, a second change appeared which was a gradual reduction in heart size. This seemed to appear first in the left ventricle, but soon the whole heart became distinctly smaller. It assumed a pendular shape which was apparently identical with that following severe hemorrhage, as determined in control experiments. This decrease in size continued during the development of the extensive pulmonary edema. During the period of asphyxial death the heart began again to enlarge, particularly the right side, and after the death plates invariably showed a dilated heart with relative increase of the right side.

Figure 7 illustrates strikingly the stages of increase and decrease in heart size. The area of the normal heart shadow as determined by the planimeter was 51.2 sq. cm. Thirty-nine minutes after gassing, the area had increased to 55.4 sq. cm. Eleven hours and fifty-three minutes after exposure the heart had decreased to 44.5 sq. cm. This was at a time when the pulmonary edema had become very marked.

*Changes in the lungs.* The condition of the lungs was determined in a large number of animals by means of physical, fluoroscopic and stereoscopic x-ray examinations. Immediately after removal from the gas chamber there was noted a diffuse clouding of the lungs usually most marked in the middle and upper lobes but sometimes involving

the lower lobes also. This was accompanied by an increase in density and number of the streaky shadows cast by the larger bronchi and vessels at the roots of the lungs.

For a few hours the cloudy appearance of the lungs generally increased slowly without however being associated with any constant physical signs. Occasionally, transitory fine dry crackling râles were heard during expiration, and occasionally there was a slight roughening of the normal respiratory sounds.

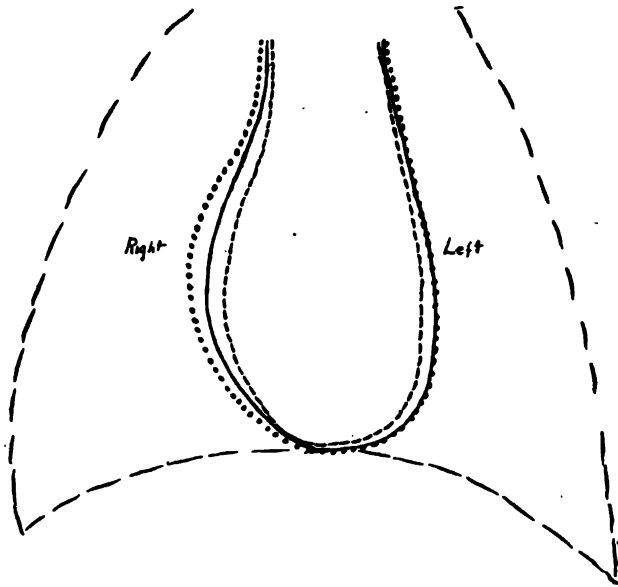


Fig. 7. Superimposed outlines of three x-ray photographs taken at intervals during phosgene poisoning to show changes in shape of the heart. The solid line indicates the normal. The dotted line shows the right-sided dilatation thirty-nine minutes after gassing. The broken line is from a photograph taken eleven hours and fifty-three minutes after exposure to the gas. The heart had then become pendular in shape and much reduced in size.

After these few hours the lungs often appeared somewhat clearer. This improvement, however, was transient and gave way during the latter third of the experiment to a streaky, mottled appearance, at first marked near the roots of the lungs but later involving the whole of both lungs. Often the heart outline was in part lost or obscured. It was at this time that numerous râles of all varieties made their appearance, medium moist predominating. These were heard in both

inspiration and expiration. In brief, the clinical signs were now those of extensive pulmonary edema and passive congestion.

In the later stages there was frequently an impairment of the percussion notes, most evident in the pendent portions, and an extension of deep cardiac dullness especially on the right side. X-ray plates showed, however, that this was not due to cardiac enlargement but probably to better transmission of heart dullness by the edematous lung.

There are thus three more or less distinct changes to be made out by means of the x-ray in the lungs of animals fatally poisoned with phosgene. First a diffuse cloudiness due probably to the initial epithelial injury and to the agglomeration of the corpuscles in the capillaries. Second, an improvement, a decrease in the cloudiness, accounted for by a reopening of many of the capillary passages. Third, a marked increase in the density and extent of all shadows cast by bronchi and blood vessels and an extension of the mottled appearance to all parts of the lungs. It is worthy of mention that it is only during this latter stage that the classical clinical signs of pulmonary edema develop. The x-ray has proved a much more delicate method of following lung lesions than the older methods of percussion and auscultation.

*Respiratory rate and pulmonary aeration.* One of the earliest results of phosgene gassing was an increase in the respiratory rate. By the end of the fourth period, that is stage 1, the rate had usually increased from an average of 30 to 45 per minute. This increase continued through stage 2 until the death period itself, when the respirations of course became irregular and gradually less rapid.

Respiratory rate, particularly in the dog where there may be much panting, gives a poor idea of the amount of air actually passing in and out of the lungs. To secure such data a series of five experiments was run in which the dogs were placed in an air-tight rigid chamber that enclosed the entire animal except the head. An inflated rubber collar secured an air-tight fit around the neck. The box was connected by tubing to a piston recorder which not only made a record of the respiratory rate but on calibration gave an accurate measure of the air passing in and out of the lungs.

The data thus secured, as shown in table 2, indicate in all cases a final marked increase in pulmonary aeration. In all cases the amount of air respired was at least doubled in the latter periods of the poisoning. Even after the break in arterial pressure when respirations often slowed down, the increase in aeration was maintained. In three cases

the amount respired immediately after gassing was definitely lower than normal.

*Temperature.* The only constant change in temperature was a gradual fall as blood concentration increased and death became imminent. This amounted to as much as 2 degrees in many cases. Very frequently there was a slight initial rise in temperature during stage 1.

*Alkaline reserve.* Our studies on alkaline reserve are very incomplete. Determinations taken at irregular intervals in eleven experiments seem however to justify the statement that there is no change of particular significance until the latter periods of the experiment. At about the time blood pressure falls so markedly there is a decided decrease in the alkaline reserve. The tissues at this time are undoubtedly suffering from oxygen want and the decrease in carbonate is due to the formation of fixed acids.

TABLE 2

*Showing pulmonary aeration during phosgene poisoning. Aeration in cc. per minute*

EXPERIMENT NUMBER	NORMAL	IMMEDIATELY AFTER GASSING	ABOUT ONE HOUR BEFORE DEATH
34	1360	420	3000
35	1265	850	2501
36	950	1540	2080
37	1175	1650	2660
38	1370	1060	3080

## DISCUSSION

The various pathological physiological studies just reported are of interest chiefly in giving us a conception of phosgene poisoning as a whole. In fatal cases, and our studies were made on such, two rather well-marked stages are apparent. The first of these is characterized by the nervous reflexes due to the irritation of the gas in the respiratory passages and by the direct chemical action of the gas or its decomposition products on the blood. The second stage is characterized by well-developed pulmonary edema and its natural consequences. The whole subject indeed might well be termed a study of pulmonary edema induced by phosgene.

The first effect of the gas is to injure the linings of the deep respiratory passages. Spasm of bronchiole musculature is evidence of the

stimulation produced by the fumes. As a result of this we have a reflex cardiac inhibition, very characteristic of many stimulations of the respiratory surfaces. The composite curve of heart rates shows a decrease during the first half of the poisoning. Another reflex from the same cause is a vasomotor one which brings about peripheral constriction with a rise in blood pressure. The x-ray gives evidence of pulmonary injury at this time although physical signs are usually entirely absent. More important than these nervous phenomena, however, is the direct action of the gas on the blood in the pulmonary capillaries. Here the red corpuscles are agglomerated into masses which largely fill and block the capillary passages. Bubbling gas through blood shows that it may have just this effect. The results of this plugging of the capillaries are twofold. In the first place pulmonary resistance is increased and a load thrown upon the right heart. Evidence of this is seen in the right cardiac dilatation found in the x-ray plates. A second result is the removal of red cells from the circulation which results in a decreased hemoglobin content of the blood. This first stage of phosgene poisoning, as shown by Underhill, is most easily determined by following the hemoglobin and it may be spoken of as the stage of decreased hemoglobin concentration.

Underhill (1) has explained the decreased hemoglobin concentration on the basis of blood dilution by body fluids. Just how or why blood volume should be increased at this time is not clear. That this interpretation is probably not sufficient we believe to be shown by direct determinations which indicate no increase in blood volume, and by the histological examinations which show the red cells agglomerated in the capillaries.

Long before the end of the first stage pulmonary edema is under way. The direct cause of this is undoubtedly the increased permeability of alveolar and capillary walls due to direct injury from the gaseous fumes. The increased pulmonary blood pressure resulting from the capillary plugging greatly favors the condition.

The second stage of acute phosgene poisoning is characterized by rapid development of the pulmonary edema with all its physical signs. This results in a decreased blood volume and increased hemoglobin concentration and a smaller diastolic size of the heart. The essential thing is the greatly reduced blood volume, which is almost entirely accounted for by the increased fluid in the lungs.

Death under such conditions may obviously be accounted for in either one of two ways. The edematous condition of the lungs may



interfere with the gaseous exchanges to such an extent that the animal asphyxiates, or the blood volume may be so reduced that even though the hemoglobin is oxygenated there is not enough fluid to secure its proper distribution to and circulation in the tissues. So far as the tissues themselves are concerned the result is the same. They die of oxygen starvation. In our opinion death is due to a combination of the two causes. Our belief is based on a series of experiments in which gassed animals were immediately placed in chambers containing 40 to 60 per cent oxygen. The  $\text{CO}_2$  content was of course kept within physiological limits and oxygen supplied automatically so as to keep the amount constant. The majority of these animals lived from 48 to 72 hours instead of the average 16 and seemed to be recovering. On being released the usual occurrence was for each dog to walk across the room and fall into an asphyxial convulsion which quickly terminated in death. Several of the animals were hurried back into the oxygen chamber and resuscitated. These animals were edematous with reduced blood volumes, but in an atmosphere of 40 per cent oxygen life was preserved, one is tempted to believe because of complete hemoglobin saturation and physical absorption of oxygen. That decreased blood volume is a cardinal part of the syndrome, and possibly by far the most important part, need not be questioned.

We do not at this time wish to discuss the treatment of phosgene poisoning further than to point out the suggestions naturally arising from our work. In stage 1 bleeding may serve to relieve the right heart and reduce pulmonic pressure. The heart would thus be in better shape for succeeding emergencies and the edema would develop less rapidly. This treatment alone might in many cases tide the animal over and make possible its recovery.

In stage 2 oxygen is of course suggested. Administration of oxygen, although of value, must be looked upon as more or less palliative. It can neither restore alveolar epithelium nor reduce the edema. Wilson and Goldschmidt (3) have recently shown in chloropicrin poisoning that oxygen administration does not prevent concentration of the blood. It may, however, make life possible until the body can take care of itself. Any treatment which would permanently increase blood volume would of course be fundamental.

## SUMMARY

1. A study of the pathological physiology of acute phosgene poisoning shows a well-marked succession of events which finally results in typical pulmonary edema. The microscope and the x-ray both show an early injury to the linings of the deep respiratory passages. Irritation from this results in a certain amount of reflex cardiac inhibition and vasoconstriction. Coincident with these changes there is a direct action of the gas on the red blood cells, which causes them to agglomerate and obstruct the pulmonary capillaries. The removal of red blood cells from the active circulation in this way results in a decreased hemoglobin percentage. The plugging of the capillaries throws a strain on the right heart and a right-sided cardiac dilatation is apparent. These are the chief characteristics of stage 1.

2. Even during stage 1 the injury to the alveolar membranes and the increased pressure have initiated the transfusion of fluid from the blood into the tissue spaces and later into the air passages of the lungs. The rapid development of this edema is the chief characteristic of stage 2. It results in hemoglobin concentration, reduction in blood volume and decrease in heart size, all three of which proceed to extreme degrees. Death ultimately results from decreased oxygenation of the pulmonary blood and from oxygen starvation of the tissues due to decreased blood volume, the latter, as Underhill states, being probably the more important.

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# TO WHAT EXTENT ARE THE PHYSIOLOGICAL EFFECTS OF CARBON DIOXIDE DUE TO HYDROGEN IONS?

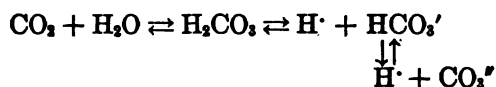
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## INTRODUCTION

Carbon dioxide in aqueous solutions—and in living organisms there is always water to form such solutions—behaves as a weak acid, dissociating in the following manner:



It is a matter of some importance to determine to what extent its well-known physiological properties are due to the presence of hydrogen ions in its solutions, i.e., how far it behaves as an ordinary acid—and to what extent they may be of a more specific nature, due possibly to some peculiarity of the undissociated  $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$  molecule.

This question is of especial interest in connection with the problem of the regulation of the respiratory movements of the higher vertebrates. The delicacy of the response of the mammalian respiratory center to changes in the  $\text{CO}_2$  tension of the alveolar air and the blood was strikingly shown by the work of Haldane and Priestley (1). Several years later Winterstein (2) suggested that it is not to the  $\text{CO}_2$ , as such, but rather to an excess of hydrogen ions in the blood, that the response occurs. This conclusion was strongly supported by the work of Hasselbalch and Lundsgaard (3), (4) who showed that while the  $\text{CO}_2$  content of the blood fluctuates considerably with different diets, etc., its hydrogen ion concentration is maintained practically constant; and this view of the rôle of hydrogen ions has been very generally accepted by physiologists, including Haldane (5). However, there have not been wanting supporters of the view that  $\text{CO}_2$  may have a specific effect which is not due to hydrogen ions in its solutions. Among these may be mentioned Lacqueur and Verzár (6), Hooker (7) and Scott (8). The evi-

dence recently presented by the last two workers is of especial interest. Hooker and his collaborators found that for the same hydrogen ion concentration of the blood, the effect on the respiratory center of the dog is much greater with a high  $\text{CO}_2$  tension than with a low one. They are inclined to attribute this result to some specific peculiarity of the  $\text{CO}_2$  molecule, either in directly stimulating the respiratory center or in increasing its irritability to hydrogen ions. Scott, by a somewhat different method, found that in the decerebrate cat increased respiration may be brought about at a hydrogen ion concentration *lower* than that of normal blood, provided that sufficient quantities of  $\text{CO}_2$  are present. He considers that probably both hydrogen ions and undissociated  $\text{CO}_2$  molecules may serve as respiratory hormones.

In view of the general importance of the question of the nature of the effects of  $\text{CO}_2$  on living protoplasm—of which its effects on the respiratory center are a particular case—it is desirable that further work should be done on material which, unlike the mammalian respiratory center, is open to direct observation. The experiments here described have been performed with this end in view on several varieties of material, including tadpoles, various protozoa, and the taste receptors of the human tongue. They may conveniently be described in the order mentioned.

#### EXPERIMENTS ON TADPOLES

Carbon dioxide in high concentrations is extremely toxic to young tadpoles of the toad (*Bufo americanus*). When placed in a quantity of distilled water saturated with  $\text{CO}_2$  at atmospheric pressure, these animals show movements for a few seconds, and then settle to the bottom of the vessel, where they remain motionless for an indefinite period. If removed to fresh water within a minute or two, they usually recover; if the exposure lasts from three to five minutes or more at room temperature, death is the result. That the effects produced are not due merely to the absence of oxygen is shown by such an experiment as the following. A quantity of water was boiled and then placed in a test-tube in which it was cooled by passing through it a stream of hydrogen which had first been conducted through an alkaline pyrogallol solution. After the current of hydrogen had flowed for ten minutes, tadpoles were quickly introduced, the tube was restoppered and the current of hydrogen again allowed to flow. The amount of oxygen available for the animals under these conditions was practically negligible; nevertheless, movements continued for over ten minutes (as compared with a few seconds

for the  $\text{CO}_2$  saturated water) and when the animals were removed at the end of fifteen minutes, all soon regained their normal activities and showed complete recovery.

To test the view that the loss of movement and death of the tadpoles when subjected to  $\text{CO}_2$  solutions are due to hydrogen ions in the medium, solutions of hydrochloric, oxalic, formic, salicylic, acetic, butyric and carbonic acids (the last a saturated solution of  $\text{CO}_2$  in distilled water) were made up to approximately the same pH (ca. 3.8-3.9) by means of the indicator, tetrabromphenolsulphonephthalein. Two tadpoles were placed in about 20 cc. of each solution in separate test tubes and the solutions were changed twice to insure the action of the full strength of the acids. The results of the experiment were that while the animals in the carbonic acid showed cessation of all movements in five seconds, those in all of the other acids were active at the end of thirty minutes. Those in butyric acid died within an hour, while the remainder lived for several hours. It appears, therefore, that the hydrogen ion concentration of a  $\text{CO}_2$  solution is not the most important factor in determining its toxicity for tadpoles, since the same concentration when produced by other acids is incomparably less effective.

The lack of dependence of the effects of  $\text{CO}_2$  on the hydrogen ion concentration of its solutions is further brought out by the following experiment. An M/4 solution of  $\text{NaHCO}_3$  was saturated with  $\text{CO}_2$  and was found by the indicator method to have a pH of approximately 6.9, i.e., it was almost neutral, the acidity being very much less than that of distilled water in which the tadpoles live for days. Nevertheless, loss of movement and death occurred in this solution in practically the same time as in the  $\text{CO}_2$  saturated distilled water. A control experiment with the  $\text{NaHCO}_3$  alone showed that it had produced no cessation of movement or visible injury in two hours. Incidentally, this last fact disposes of the possibility that  $\text{H}_2\text{CO}_3$  might act chiefly through its anion,  $\text{HCO}_3^-$ , since this ion is present in abundance in bicarbonate solutions, which are seen to be practically without effect. The conclusion must therefore be drawn that for certain concentrations of  $\text{CO}_2$ , characteristic effects will be produced not merely at a pH which in the case of other acids is only very slightly effective, but even when the reaction of the medium is practically neutral. So far as toad tadpoles are concerned,  $\text{CO}_2$  appears to be a substance of very different properties from ordinary acids. A discussion of the possible reasons for this difference may be reserved until after a description of the experiments made upon protozoa.

## EXPERIMENTS ON PROTOZOA

Of over a dozen species of ciliate and flagellate protozoa studied by the author (9), all are killed when exposed to a current of  $\text{CO}_2$  in an Engelmann gas chamber, the time required varying greatly with different forms. *Coleps*, for example, may be killed in two or three minutes, *Paramecium bursaria* usually in ten or fifteen, while under the same conditions *Paramecium caudatum* may survive an hour or more and *Colpidium* several hours. These relatively great differences in the resistance of the different forms furnish a means of further testing the possible mode of action of  $\text{CO}_2$ . If the effects of this substance are really due to the hydrogen ions in its solutions, then approximately the same order of resistance ought to be obtained with solutions of other acids, especially when the hydrogen ion concentrations are nearly the same.

Such, however, proves not to be the case. Forms which have a high resistance to  $\text{CO}_2$  may have a low one to other acids, and vice versa. For example, the author (9) has shown that *Euplotes patella* is considerably more sensitive to  $\text{CO}_2$  than *Paramecium caudatum*. Under comparable conditions, where the latter survives for an hour or more, the former may succumb in perhaps thirty minutes; i.e., its resistance is only approximately half as great. In the case of over a dozen other acids, however, recently studied by Miss Collett (10), the exact reverse is true. "At whatever concentration they are tested, no matter what the acid, *Paramecium* is much less resistant than *Euplotes*." The author can confirm from his own personal observations the high resistance of *Euplotes*, as compared with *Paramecium*, to mineral and to a number of organic acids. Only in the case of carbonic acid does this relation fail to hold, indicating the possession by this substance of certain unique properties not shared by the other acids.

Another example is furnished by the three common species of *Paramecium*: *P. caudatum*, *P. aurelia* and *P. bursaria*. To  $\text{CO}_2$ , the order of resistance, under comparable conditions is:

$$P. caudatum > P. aurelia > P. bursaria$$

In one experiment where all three forms were found in the same culture and had, therefore, for several weeks been under the same conditions, the average times of death when exposed to  $\text{CO}_2$  were: *P. caudatum*, thirty-two minutes; *P. aurelia*, eighteen minutes; *P. bursaria*, six minutes. To N/10,000 HCl, the corresponding times in minutes were:

nine, five and twelve minutes respectively. In general, it has been found that for mineral acids the order of resistance is:

*P. bursaria* > *P. caudatum* > *P. aurelia*

That is to say, the form that is the most resistant to the mineral acids, which almost certainly act chiefly through their hydrogen ions, is *least* resistant to CO<sub>2</sub>.

Still another example is furnished by *Coleps* which, while by far the most susceptible to CO<sub>2</sub> of the forms studied, is by no means unusual with respect to mineral acids, having approximately the same order of resistance as *Paramecium caudatum*. A number of other similar cases could be mentioned. Indeed, the first half-dozen forms compared showed almost an exact inverse relation between their susceptibility to CO<sub>2</sub> and to H<sub>2</sub>SO<sub>4</sub>. This probably was merely a coincidence, since other forms studied later did not show the same relation, but the fact comes out clearly that while different forms tend to agree in the order of their resistance to other acids, their resistance to CO<sub>2</sub> is apparently an unrelated quantity. It would appear, therefore, that as a toxic agent to the protozoa studied, carbonic acid is in a class by itself.

Further evidence on this point is furnished by the visible effects of CO<sub>2</sub> on certain other protozoa. A particularly striking case is that of *Peranema*, a common flagellate with a single flagellum and a body capable of being drawn into a great variety of shapes by the contractile myonemes with which it is provided. The author has previously pointed out (9) that the effect of CO<sub>2</sub> on *Peranema* is to cause an almost instantaneous paralysis of the myonemes, while leaving the flagellum free to beat for perhaps three-quarters of an hour. If this rather striking effect is due to hydrogen ions in the CO<sub>2</sub> solution, it should be possible to imitate it with acids like HCl or H<sub>2</sub>SO<sub>4</sub> of the proper concentration. Attempts to do so, however, have shown that the effects of these acids are not only not similar, but are in some respects the reverse of those of CO<sub>2</sub>. When exposed to them, at least if the concentration be sufficiently high, the flagellum usually stops beating, but the myonemes, instead of being paralyzed, are powerfully stimulated and the animals may make vigorous contortions for an hour or more. The failure to paralyze the myonemes, but the tendency rather to stimulate them was observed at all strengths of the acids employed from N/100 to less than N/10,000. At the last mentioned concentration it was possible for the flagellum to beat, but not normally.

Very similar effects were obtained with *Euglena*, a related green form, and with the ciliate *Vorticella*. In the latter, the contractile filament of the stalk is quickly paralyzed by  $\text{CO}_2$ , while the membranelles continue to beat for a long time, whereas in various strengths of  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  the stalk is unusually active, almost up to the time of death, while the bell is for the most part closed and the activity of the membranelles clearly depressed. It appears therefore that while contractile structures like the myonemes of *Peranema* and *Euglena* and the contractile filament of *Vorticella* are quickly paralyzed by  $\text{CO}_2$ , long before other portions of the cell are inactivated, the effect of mineral acids is to stimulate them to abnormal activity almost up to the time of death of the animal. The general effects of carbonic acid, therefore, are not only quantitatively, but qualitatively different from those of other typical acids.

#### THE TASTE OF CARBON DIOXIDE SOLUTIONS

The results so far described leave little room for doubt that carbonic acid is different in its mode of action from the other acids studied. As to the reasons for this difference, there are several possible explanations. Leaving out of consideration, for the reasons already mentioned, the possibility that its peculiarities may be due to the anion  $\text{HCO}_3^-$ , there remain two plausible hypotheses: *a*, there may be some chemical peculiarity of the  $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$  molecule as a whole which is responsible for its apparently specific action; or *b*, carbonic acid, once inside a cell, may behave essentially as other acids, but may differ from them in its much greater powers of penetrating not only cell membranes, but all other cell structures as well. To decide between these two hypotheses is not as simple a matter as it might appear to be. One possible method of testing the truth of the second one would be by the use of cells containing a natural indicator sensitive to carbonic acid. Unfortunately, the author has not as yet been able to secure material suitable for the employment of this method, though he hopes soon to be able to obtain it. Another method would be to place cells or tissues in a mixture of bicarbonate and carbonic acid and by means of indicators, or otherwise, to test the penetration of the latter, by noting any rise in the alkalinity of the mixture. There are certain practical difficulties, some obvious and some not so apparent, that have to the present time prevented the successful employment of this procedure. A third method, however, of extreme simplicity, has yielded definite results. It involves the use of certain taste receptors of the human tongue.



The connection between the sour taste of acids and the presence of hydrogen ions in their solutions has long been known. Over twenty years ago Richards (11) showed that surprisingly accurate titrations could be made by the sense of taste. In general, the greater the degree of dissociation of an acid, the more pronounced the sour taste. However, the concentration of hydrogen ions is not the only factor; acetic acid, for example, has a more powerful effect, as compared with mineral acids, than its hydrogen ion concentration would lead one to expect. The question of penetration probably plays an important part in producing the observed effects. Crozier (12) points out that the order of effectiveness, in giving a sour taste, of the series of acids studied by Becker and Herzog (13) is almost identical with that which he obtained in studying the order of penetration of acids into the pigmented cells of *Chromodoris zebra* (14). It is probable, therefore, that the two important factors in determining the sourness of a solution of an acid are *a*, the actual concentration of hydrogen ions in the solution, and *b*, the penetrating powers of the acid itself.

From these considerations it is apparent that the taste of CO<sub>2</sub> solutions might be utilized in throwing light on its powers of penetrating cells and on certain of its other physiological peculiarities. The first experiments made with this end in view rather favored the theory that the more striking effects of CO<sub>2</sub> are of a specific nature, and are not primarily due to hydrogen ions. The taste of a saturated solution of CO<sub>2</sub>, as is well known, is not markedly sour; the most prominent effect in the mouth is the production of a stinging or a tingling sensation, which is entirely different from the sour taste of weak solutions of other acids. Furthermore, when such a saturated solution is diluted with distilled water to ten times its original volume, the sour taste disappears completely, though the tingling sensation is still perceptible. It might seem reasonable to conclude, therefore, that a carbonic acid solution has two effects: a weak one due to its hydrogen ions and a stronger one due to some other peculiarity of its molecules.

Such a conclusion, however, is unwarranted. If drops of a saturated solution of CO<sub>2</sub> be placed with a pipette on different parts of the tongue, or if a current of CO<sub>2</sub> from a generator be similarly directed, it appears that different parts of the tongue respond differently. On its tip the stinging sensation alone is perceived; along its sides and dorsally toward its base only the sour taste. The same relation holds for dilute solutions which, when taken into the mouth in the ordinary way, give no sour taste. Furthermore, an acid like HCl in concentrations of N/100 or

higher, when placed on the tip of the tongue, is not sour but gives the same stinging sensation that is obtained with  $\text{CO}_2$ . So far as its taste is concerned, therefore, carbonic acid does not appear to be qualitatively different from other acids; the differences are merely quantitative, and might reasonably be correlated with a more ready penetration of the taste receptors by this substance.

For purposes of comparison it might be of interest to state that according to Crozier (12) the hydrogen ion concentration of a solution of  $\text{HCl}$  which is just perceptibly sour ( $\text{N}/900$ ) is  $0.00119\text{N}$  and of the corresponding acetic acid solution ( $\text{N}/200$ )  $0.00035\text{N}$ . Calculation shows that a solution of  $\text{CO}_2$  one-tenth saturated at room temperature, in which a slightly sour taste can be detected on the proper part of the tongue, should have a hydrogen ion concentration of approximately  $0.00003\text{N}$ ; i.e., carbonic acid is about ten times as effective as acetic acid in giving a sour taste.

The hypothesis that  $\text{CO}_2$  owes its unusual properties not to the hydrogen ion concentration of its solutions but rather to its great penetrating powers and to the hydrogen ion concentration produced within the cells, was tested by the following experiment. An  $\text{M}/2$   $\text{NaHCO}_3$  solution was saturated with  $\text{CO}_2$  and found by the indicator method to have a pH of approximately 7.4; i.e., it was on the alkaline side of neutrality. Nevertheless, such a solution on the sides and the posterior dorsal portion of the tongue gave a *distinct sour taste*, which was very apparent on alternately testing it and the original unsaturated bicarbonate solution, the latter having in comparison a decidedly flat taste.

The rather surprising result of an alkaline solution with a sour taste may plausibly be explained on the assumption that the  $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$  readily penetrate the taste cells while the  $\text{NaHCO}_3$  does not. In the external medium, the dissociation of the carbonic acid is strongly depressed by the bicarbonate; within the cells this restraint is no longer effective, and the hydrogen ions are free to produce their typical effect on the taste receptors. What is true in this case might very plausibly be supposed to be true also in the case of the respiratory center; i.e., the effective hydrogen ion concentration might be very different from that of the blood, provided that the acid concerned is the quickly penetrating carbonic acid. According to this theory, the results of Hooker and of Scott are readily intelligible and do not necessarily conflict with the orthodox view that hydrogen ions are the respiratory hormone. In one sense (i.e., in its unique powers of penetration)

CO<sub>2</sub> might be looked upon as having a specific effect, though acting within the cells like other acids by means of hydrogen ions.

The general mode of action of CO<sub>2</sub> may be pictured as follows. In the medium surrounding the cell are H<sup>+</sup>, HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>=</sup> ions, and CO<sub>2</sub> and H<sub>2</sub>CO<sub>3</sub> molecules. The ions, as such, do not penetrate to any appreciable extent, though the H ions, at least, acting on the cell surface, may have a stimulating effect. However, one or the other, or both, of the undissociated molecules may readily penetrate to all parts of the cell and there dissociate in the ordinary way giving hydrogen ions which produce effects not obtained at all with corresponding concentrations of other acids. A somewhat analogous case is perhaps furnished by "mustard gas." The usual explanation of the method of action of this substance is that in aqueous solutions it becomes hydrolyzed to HCl and dihydroxyethylsulphide; the HCl, at least in weak solutions, has very feeble powers of entering cells and an old solution is therefore relatively non-toxic. The unaltered molecules in a fresh solution, however, readily enter cells and when hydrolysis occurs within them, the HCl set free may have strongly toxic effects which may be imitated by the direct injection of this substance with a very fine pointed pipette (15). Whether or not this simple explanation is adequate to account for all of the peculiarities of mustard gas, it at least may be used to illustrate the possible mode of action of CO<sub>2</sub>.

The similarities and differences between carbonic and other acids may be brought out very clearly by studying their visible effects on such an organism as *Paramecium*. A mineral acid, such as HCl or H<sub>2</sub>SO<sub>4</sub>, clearly acts from without inward, killing or injuring its way as it goes. One of the first effects produced by it is on the cilia, locomotion ceasing and their beat becoming slow and irregular. At a time when the beat of the cilia has almost ceased, the internal structures in the cell may be apparently unaffected. The contractile vacuoles may pulsate regularly, the protoplasm be clear and transparent and the nuclei possess their normal appearance. As the acid slowly enters the cell, the pulsations of the contractile vacuoles cease, swelling of the body occurs, the nuclei stand out sharply from the cytoplasm, and the latter becomes coagulated and opaque. With carbonic acid the results are very different, this substance acting in a sense from within outward. Animals exposed to it in the usual way in an Engelmann gas chamber quickly show visible internal signs of its effects. Swelling of the body begins almost immediately, and may continue until the buccal groove is almost obliterated; the nuclei soon stand out sharply, as when

treated with acetic acid; the contractile vacuoles become paralyzed; and even signs of coagulation of the protoplasm may appear—all before the cilia are sufficiently affected to bring about cessation of locomotion. Evidently the  $\text{CO}_2$  enters immediately, the mineral acid much more slowly; though the final effects produced in the two cases are not dissimilar.

The differences between the effects of  $\text{CO}_2$  and of ordinary acids on *Peranema*, *Vorticella*, etc., described above, may readily be explained by the greater penetrating powers of  $\text{CO}_2$ . It is not surprising, in the light of the facts already mentioned, that it is able to paralyze so quickly the myonemes of *Peranema* and *Euglena* and the contractile filament of the stalk of *Vorticella* (all of which are internal structures) while concentrations of mineral acids strong enough to stop the beat of the flagella and the membranelles are unable to do so, and manifest their presence for a long time merely by their stimulating action on the surface of the cell. The different order of resistance of different protozoa to  $\text{CO}_2$  and to an acid such as  $\text{HCl}$  is also exactly what would be expected when it is remembered that the action of  $\text{CO}_2$  is primarily internal and that of the other acid is primarily external and only secondarily internal. The results obtained with protozoa, therefore, do not conflict with the view here advanced as to the nature of the action of  $\text{CO}_2$ .

In conclusion it may be said that the general result of the experiments described in this paper is to indicate that while in certain respects  $\text{CO}_2$  in aqueous solutions behaves as an ordinary acid, acting through its hydrogen ions, in other respects—especially in its remarkable powers of penetrating living cells—it may be said to have a specific action different from that of other acids. This theory makes it possible to reconcile what have been until now two apparently contradictory points of view as to the mode in which it produces its characteristic effects on the respiratory center of mammals, and on living protoplasm in general.

#### SUMMARY

1. A saturated solution of  $\text{CO}_2$  is incomparably more toxic to toad tadpoles than are solutions of hydrochloric, oxalic, salicylic, formic, acetic or butyric acids of the same hydrogen ion concentration.
2. In the presence of sufficient bicarbonate to give practically a neutral reaction to the solution, the toxic properties of  $\text{CO}_2$  to tadpoles are unchanged.

3. The order of resistance of various protozoa to  $\text{CO}_2$  bears no relation to the order found in the case of other acids.

4.  $\text{CO}_2$  very quickly paralyzes contractile structures in protozoan cells while causing little injury to flagella, etc. Under certain conditions mineral acids may produce apparently exactly the opposite effects.

5. The differences between carbonic and the other acids studied may probably be accounted for by a difference in their powers of penetrating living cells.

6. Due possibly to the ready penetration of the taste receptors by  $\text{CO}_2$ , and to its subsequent dissociation, an alkaline bicarbonate-carbonic acid mixture may have a distinct sour taste.

7. The hypothesis that similar conditions exist in the mammalian respiratory center would reconcile the two apparently conflicting views held at present as to the method in which  $\text{CO}_2$  acts as a respiratory hormone.

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## THE GASTRIC RESPONSE TO FOODS<sup>1</sup>

### VII. THE RESPONSE OF THE NORMAL HUMAN STOMACH TO VEGETABLES PREPARED IN DIFFERENT WAYS

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The object of this investigation was to determine the response of the normal human stomach to the different members of the class of foods grouped together under the name of vegetables. These comprise a variety of leaves, stems, roots, bulbs, tubers and seeds, differing widely in chemical composition and valued in the diet for different reasons. An attempt was made to study the digestion of members of each of these classes. Wherever practicable comparisons of the raw and boiled vegetables were carried out and different methods of cooking were also employed.

Beaumont, in connection with his experiments on Alexis St. Martin, studied the digestion of certain vegetables in the stomach of his subject. Definite quantities of foods were not given in his test meals. These meals were also in most cases of a mixed character. Beaumont's results are given in table 1.

The results recorded by Beaumont agree, in hardly any respect, with the findings in our more carefully controlled experiments. Where Beaumont found a difference of an hour in favor of baked over boiled potatoes, we found no such advantage. Beaumont found boiled potatoes to remain in the stomach  $3\frac{1}{2}$  hours. We found 100 grams of boiled potatoes to remain only  $2\frac{1}{4}$  hours in the case of a man of slow-emptying type. Beets we found to leave the stomach rapidly (in from 1 to 2 hours). Beaumont found them to remain long ( $3\frac{3}{4}$  hours). Parsnips we found to require distinctly longer than carrots or beets.

<sup>1</sup> The expense of this investigation was defrayed by contributions from Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

Beaumont's results would indicate that the reverse was true. Beaumont gives  $3\frac{1}{2}$  hours for boiled turnips; we found  $1\frac{1}{2}$  hours. Beaumont found boiled cabbage to require 2 hours longer than raw. We found no such difference. Beaumont's figures would indicate that boiled rice and barley leave the stomach in a fraction of the time required for other vegetables. This finds no support in our experiments. This is not a criticism of Beaumont's valuable pioneer work but only of the uncritical use of his data.

Penzoldt (2) made a number of experiments on a single subject (Croce). In most cases he gave 150 gram portions (based on the raw weight). Samples were removed at half-hour periods. Hence no closer

TABLE 1  
*Digestion of vegetables in the human stomach (Beaumont)*

FOOD	EVACUATION TIME
	<i>hours and minutes</i>
Potatoes, boiled.....	3:30
Potatoes, roasted.....	2:30
Potatoes, baked.....	2:30
Beets, boiled.....	3:45
Carrots, boiled.....	3:15
Parsnips, boiled.....	2:30
Turnips, boiled.....	3:30
Green corn and beans.....	3:45
Cabbage, raw, plain.....	2:30
Cabbage, raw, with vinegar.....	2:00
Cabbage, boiled.....	4:30
Rice, boiled.....	1:00
Barley, boiled.....	2:00

time comparisons were possible. The drinking of water by Penzoldt's subjects must also be considered, and the fact that most of his results are based on single experiments. The results obtained by this author are given in table 2.

The results of Penzoldt show a fair agreement with our own in the few cases where they are directly comparable.

The method of investigation used in our experiments was that previously described (3). More emphasis was laid on the microscopical examinations of samples which were made in nearly all cases. The subjects were normal medical students and laboratory workers who were at rest during the experimental period.

The results as regards gastric acidities developed and evacuation times are given in table 1. The distinction between subjects of rapid- and slow-emptying types is less in the case of most vegetables than in the case of meats. The classification of subjects is based upon the response to other foods as well as vegetables.

Comparative responses of the same individuals to different forms of vegetables are charted in figures 1 to 31. In these figures the prime or accent marks indicate curves of free acidity.

TABLE 2  
*Digestion of vegetables in the human stomach (Penzoldt)*

FOOD	AMOUNT	EVACUA- TION TIME
	grams	hours and minutes
Potatoes, boiled.....	150	2:05
Potatoes, mashed.....	150	2:45
Potatoes, "Gemüse".....	150	3:20
Carrots, boiled.....	150	3:20
Radishes, raw.....	150	3:05
Lentils, ground and acidified.....	150	4:05
Peas, boiled.....	200	4:15
Beans, string, boiled.....	150	4:20
Kohlrabi, boiled.....	150	3:05
Cauliflower, "Gemüse".....	150	2:35
Cauliflower, boiled.....	150	2:25
Cauliflower, vinegar and oil.....	150	2:05
Asparagus, boiled.....	150	2:45
Asparagus, oil and vinegar.....	150	3:00
Spinach, boiled.....	150	3:30
Cucumbers as salad.....	150	3:15
Rice, boiled.....	100	3:35

*Potatoes, whole boiled, baked, mashed and fried.* Boiled whole white potatoes left the stomach rather quickly requiring  $2\frac{1}{4}$  hours for a slow-emptying man (fig. 1). The same man required  $1\frac{1}{2}$  hours for creamed potatoes. For baked potatoes, plain, he required  $2\frac{1}{4}$  hours but baked potatoes with butter remained  $3\frac{1}{4}$  hours. Another subject (fig. 11) required  $1\frac{1}{2}$  hours for creamed potatoes and but 15 minutes longer for baked potatoes with butter. It appears therefore that boiled potatoes and creamed potatoes are handled in about the same time as the plain baked potatoes but that the addition of butter causes a slight delay. A potato salad required 2 hours as compared with  $2\frac{1}{4}$



hours for mashed potatoes, on one subject (figs. 3 and 4) while in the case of a slow-emptying type of stomach 3 hours were found necessary for the mashed potatoes and  $3\frac{1}{2}$  hours for the salad (fig. 5). Plain mashed potatoes were compared with mashed potatoes to which milk and butter had been added (figs. 3 and 5). In one case these additions delayed evacuation 15 minutes, in the other they hastened it 15 minutes. The influence of the milk and butter hence cannot have been marked.

Fried potatoes are frequently stated to be more difficult of digestion than boiled or mashed potatoes. We were, therefore, somewhat surprised to find that on the average fried potatoes left the stomach fully as rapidly as potatoes cooked in other ways (see figs. 4, 5, 6, 7 and 8). One subject (figs. 3 and 4) required  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours for fried potatoes as compared with 2 to  $2\frac{1}{2}$  hours for mashed potatoes and potato salad. Another subject (fig. 5) required a little longer for French fried potatoes than for mashed potatoes but no longer than for potato salad. Subject McEl (figs. 6 and 7) showed an emptying time of  $2\frac{1}{4}$  hours for plain or German fried potatoes as compared with  $1\frac{3}{4}$  hours for boiled and 2 hours for baked sweet potatoes. Subject Mil (fig. 8) required  $2\frac{1}{4}$  hours for either French fried or boiled potatoes.

The order of rapidity of digestion of different kinds of fried potatoes on one subject (figs. 3 and 4) was German fried, first, plain fried, second and French fried, third. Another subject showed exactly the same response to German fried and plain fried potatoes. It is possible that the French fried potatoes require a few minutes longer than the others but the difference cannot be marked.

Potato chips were also compared with French fried potatoes. Because of their high content of dry matter, especially fat, only 50 grams of the chips were fed. They required  $2\frac{1}{2}$  hours to digest as compared with  $2\frac{1}{4}$  hours for the fried potatoes (fig. 8). This would indicate that potato chips are handled rather readily by the stomach considering their fat content of about 40 per cent.

*Sweet potatoes, dasheens and cassava roots.* Boiled sweet potatoes remained in the stomach  $2\frac{3}{4}$  hours as compared with  $2\frac{1}{4}$  hours for boiled white potatoes (figs. 1 and 2). Baked sweet potatoes with butter required  $2\frac{3}{4}$  hours as compared with  $1\frac{3}{4}$  hours for baked white potatoes with butter (fig. 11). Plain fried sweet potatoes required, with the same subject, 2 hours. Sweet potatoes in general therefore appear to be retained in the stomach longer than white potatoes cooked in the same way.

Dasheens were cooked in several ways, namely, boiled, baked, riced, plain fried and French fried. The boiled dasheen required, for a subject of slow-emptying type (see fig. 5)  $3\frac{1}{2}$  hours as compared with  $2\frac{3}{4}$  to  $3\frac{1}{2}$  hours for white potatoes prepared in different ways. In another case baked dasheens required  $2\frac{3}{4}$  hours as compared with  $2\frac{1}{4}$  hours for baked white potatoes and  $2\frac{3}{4}$  hours for boiled sweet potatoes (fig. 2). A third subject (see figs. 3 and 4) showed an evacuation time of 3 hours for baked dasheen as compared with  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours for white potatoes prepared in different ways and  $3\frac{1}{2}$  hours for baked sweet potatoes with butter. Riced dasheen took a shorter period of time,  $2\frac{1}{4}$  hours, as compared with  $1\frac{1}{4}$  to  $2\frac{1}{4}$  hours for white potatoes and 2 hours for baked sweet potatoes (see figs. 6 and 7). French fried dasheens took distinctly longer to leave the stomach than white potatoes prepared in the same way (see fig. 8). Another subject of the rapid-emptying type showed very little difference in the emptying time of dasheens as compared with potatoes (see fig. 11). In general dasheens appear to require a longer period of gastric digestion than white potatoes but no longer than sweet potatoes.

Cassava root was tested in the boiled and baked forms. A subject of the rapid-emptying type showed an evacuation time for boiled cassava of  $2\frac{1}{2}$  hours (see table 3). A subject of the slow-emptying type required 3 hours as compared with  $2\frac{1}{4}$  hours for boiled white potatoes (figs. 1 and 2). Baked cassava with butter required  $2\frac{3}{4}$  hours as compared with  $1\frac{3}{4}$  to  $2\frac{1}{4}$  hours for boiled or fried white potatoes (figs. 6 and 7). The cassava root thus requires a little longer to digest in the stomach than white potatoes similarly prepared.

Boiled oyster plant required about the same period of gastric digestion as white potatoes (see fig. 11).

*Beets, carrots, parsnips, radishes and turnips.* Beets whether plain boiled or pickled left the stomach rapidly, that is, in from 1 to  $1\frac{1}{2}$  hours for subjects of the rapid type and 2 hours for a subject of the slower type (see figs. 9, 13, 24 and 10). Pickling reduced the time required for beets to leave the stomach (fig. 13). This may be due to the acidity of the pickled beets.

Carrots were fed raw and boiled. The raw carrots left rapidly, that is, in from  $1\frac{1}{2}$  to 2 hours (figs. 14 and 15). Boiled carrots took the same subjects distinctly longer, that is,  $2\frac{1}{4}$  to  $2\frac{1}{2}$  hours. This may be due to the greater acid-combining power of the boiled carrots, as is indicated by the much higher acid curves obtained with the boiled vegetables. These results do not confirm the statement of Hutchison

that carrots are not easily disposed of by the stomach (4), though it is true the cellular masses disintegrate slowly.

Boiled parsnips were tested on two subjects. In each case these vegetables remained in the stomach 3 hours (figs. 14 and 24). Parsnips thus required distinctly longer than beets or carrots. This may be because parsnips are more irritating to the mucosa of the intestine.

Radishes were given in 50 gram portions to two subjects. In one case (figs. 15 and 16) the radishes left the stomach in  $1\frac{1}{2}$  hours or much more rapidly than 100 grams of carrots or sugar corn, etc. The acidity developed was low in this case but moderate in the other subject (fig. 10). In the latter case the radishes left in  $1\frac{1}{2}$  hours or more quickly than any vegetables except red beets.

An alcoholic extract of the radish peel furnishes a very sensitive indicator for acid and alkali titrations as pointed out by Sacher (3) whose results we were able in a general way to confirm. As the pink color of the acid solution changes to blue in an hydroxyl-ion concentration less than that required for phenolphthalein it did not interfere appreciably with our titrations for total acidity.

Boiled turnips left the stomach rapidly. In both cases (figs. 9 and 12) 100 grams of turnips left in  $1\frac{1}{2}$  hours. This may be attributed to the low content of turnips in substances combining with acid.

*Beans, peas, endives and lentils.* Boston baked beans were tried out on two individuals (see figs. 17 and 23). As might be expected from their high protein content, these beans remained somewhat longer in the stomach than most other forms of vegetables and developed higher combined acidities. Thus where beans required  $3\frac{1}{2}$  hours, cabbage raw or boiled required  $1\frac{1}{2}$  hours (fig. 23). Baked lima beans were tested on one subject of the rapid-emptying type and while only 2 hours were required by this subject to digest the beans, a shorter period was required for nearly all of the other vegetables tested (see figs. 11 and 12).

String beans, on the other hand, left the stomach rapidly resembling the green vegetables in their stimulatory power upon the stomach (see figs. 8, 9 and 18). Any view that string beans normally remain a long time in the stomach is therefore shown to be without foundation.

Boiled peas left the stomach in  $1\frac{1}{2}$  hours in each of the two cases where they were fed. In each case they were handled more rapidly than beans (figs. 11, 12 and 17).

But a single experiment was carried out on boiled lentils (see fig. 21). These required  $2\frac{1}{2}$  hours to leave the stomach or a little longer than

TABLE 3  
Response of the human stomach to vegetables

NUMBER	SUBJECT	FOOD AND PREPARATION	RAPID-EMPTYING TYPE			SLOW-EMPTYING TYPE		
			Evacuation time, hours and minutes	Highest total acidity	average	Evacuation time, hours and minutes	Highest total acidity	average
1	Ev	Potatoes, whole, boiled.....				2:15	113	113
2	Ev	Potatoes, creamed.....				2:15	107	107
3	Lei	Potatoes, creamed.....	1:30	105	105	1:45	108	108
4	Ev	Potatoes, baked, plain.....				2:15	108	108
5	Lei	Potatoes, baked, and butter.....	1:45	88	88			
6	Mi	Potatoes, baked, and butter.....	2:15	91	90	3:15	118	118
7	Ev	Potatoes, baked, and butter.....	2:00	99	99	3:30	109	109
8	Sok	Potatoes, salad.....	2:00	99	99	3:00	120	120
9	Dur	Potatoes, salad.....	2:15	97	97	3:00	93	93
10	Sok	Potatoes, mashed, plain.....	2:15	97	97	2:15	72	72
11	Dur	Potatoes, mashed, plain.....	2:30	89	89	2:45	96	96
12	Sok	Potatoes, mashed, with milk and butter.....	2:30	89	89	2:15	105	105
13	Dur	Potatoes, mashed, with milk and butter.....				3:45	122	122
14	McEl	Potatoes, plain, fried.....	2:00	106	106	2:15	96	96
15	Sok	Potatoes, plain, fried.....	2:15	41	41	2:45	105	105
16	Mi	Potatoes, French fried.....	2:30	92	67	3:45	122	122
17	Sok	Potatoes, French fried.....	2:30	92	98	2:15	96	96
18	Dur	Potatoes, French fried.....	1:30	98	55	2:45	105	105
19	McEl	Potatoes, German fried.....	2:30	55	55			
20	Sok	Potatoes, German fried.....	1:30	98	98	2:45	105	105
21	Mi	Potato chips.....	2:30	55	55	3:15	124	124
22	Ev	Sweet potatoes, boiled.....	2:45	56	56	2:45	118	118
23	Lei	Sweet potatoes, baked, and butter.....	2:00	41	41			
24	Lei	Sweet potatoes, plain, fried.....						
25	Dur	Dasheen, boiled.....						
26	Ev	Dasheen, baked.....						



TABLE 3—*Concluded*

NUMBER	SUBJECT	FOOD AND PREPARATION	RAPID-EMPTYING TYPE			SLOW-EMPTYING TYPE		
			Evacuation time, hours and minutes		Highest total acidity	Evacuation time, hours and minutes		Highest total acidity
			average	average		average	average	
61	Wha	Asparagus, canned.....	1:30	1:30	36	1:15	63	
62	Fla	Cabbage, raw.....	1:15	1:15	79	2:00	98	
63	Em	Cabbage, raw.....				1:45	90	84
64	Daly	Cabbage, raw.....				3:00	119	119
65	Rud	Cabbage, raw (250 grams).....						
66	Rud	Cabbage, boiled.....	1:15	1:15	62	1:30	61	61
67	Fla	Cabbage, boiled.....				2:00	118	118
68	Ema	Cold slaw.....	2:15	2:15	63	2:00		
69	Fla	Sauerkraut and vinegar.....	1:45	1:45	92	3:30	79	79
70	Ema	Cauliflower, boiled.....				3:00	91	91
71	Wha	Cauliflower, boiled.....	3:00	3:00	79			
72	Ema	Celery, raw.....	1:45	1:45	96	2:45	84	84
73	Grab	Celery, raw.....				2:15	72	72
74	Mat	Celery, creamed.....	2:00	2:00	41	3:00	48	48
75	Mat	Celery, creamed.....	1:45	1:45	56	2:45	103	103
76	Grab	Celery, creamed.....				3:15		
77	Fla	Lettuce, raw, plain.....	2:00	2:00	41			
78	Ema	Lettuce, raw, plain.....	1:45	1:45	56			
79	Fla	Lettuce, sugar and vinegar.....						
80	Fla	Lettuce, olive oil and vinegar.....	2:00	2:00	92	3:00	48	48
81	Ema	Lettuce, olive oil and vinegar.....	1:45	1:45	82	2:45	103	103
82	Ev	Spinach, boiled.....				3:15		
83	McEl	Spinach, boiled.....	2:00	2:00	60			
84	Sok	Spinach, boiled.....	1:45	1:45	71			
85	O'Br	Onions, stewed.....	2:45	2:45				
86	Wha	Onions, stewed.....						

			1:30	1:30	50	50	2:30	2:30	78	78
87	Gold	Onions, fried (25 grams)	1:30	1:30	50	50	2:30	2:30	78	78
88	Wil	Onions, fried (25 grams)	1:15							
89	Lei	Chayotes, creamed	1:30		70					
90	Kru	Chayotes, creamed	2:00		22					
91	Mi	Chayotes, creamed	2:00	1:40	51					
92	Wat	Chayotes, creamed	2:00	1:45	59	50				
93	Get	Cucumbers, raw, plain	1:45	1:45	71	71	3:15	3:15	58	58
94	Kiel	Cucumbers, raw, plain	1:30	1:30	43	43	3:00	3:00	39	39
95	Get	Cucumbers, raw, salted	2:15		97					
96	Kiel	Squash, boiled	2:30	2:25	97	97				
97	Kru	Squash, boiled	1:00		30					
98	Wat	Tomatoes, raw	1:15	1:10	49					
99	Kru	Tomatoes, raw				40				
100	Wat	Tomatoes, raw					2:00	2:00	84	84
101	Mal	Tomatoes, raw					1:45	1:45	54	54
102	Kiel	Tomatoes, stewed, canned					2:30	2:30	35	35
103	Mal	Tomatoes, stewed, canned					3:30	3:00	50	42
104	Kiel	Tomatoes, stewed, canned					3:00	3:00	93	93
105	Son	Mushrooms, sauté (50 grams)	1:45	1:45	100	100				
106	Far	Mushrooms, sauté (50 grams)								
107	Mur	Mushrooms, sauté (100 grams)	2:15		95					
108	Isr	Mushrooms, sauté (100 grams)	2:15	2:15	89	92				
109	Mir	Mushrooms, sauté (100 grams)	1:30	1:30	53	53	3:00	3:00	60	60
110	Col	Barley, boiled	2:30		79					
111	Kru	Barley, boiled	1:45	2:10	91					
112	Wat	Corn, sugar, canned	2:00	2:00	74		2:30	2:30	46	46
113	Kiel	Corn, sugar, canned								
114	Kara	Hominy, boiled					2:30	2:30	51	51
115	McG	Rice, boiled, unpolished					3:15	2:45	80	80
116	Col	Rice, boiled, unpolished					2:30	2:45	8	56
117	Lea	Rice, boiled, unpolished					1:45	1:45	72	72
118	Ev	Rice, boiled, polished					4:00	2:50	39	55
119	Lea	Rice, boiled, polished					1:30	1:30	42	42
120	McG	Agar-agar								

\* Acidities are expressed as cubic centimeters of N/10 alkali required to neutralize 100 cc. of sample.

peas. Raw endives required  $1\frac{3}{4}$  to 2 hours in the cases of two individuals of the rapid-emptying type. They thus resembled peas as far as evacuation time was concerned (see figs. 19 and 20).

*Cabbage, lettuce, asparagus and cauliflower.* Asparagus (a canned variety) left the stomach rapidly, requiring but  $1\frac{1}{2}$  hours (see fig. 22). Boiled cauliflower required for the same subject a few minutes longer. In the case of a subject of a different type (see fig. 24) the cauliflower took as long as baked beans and much longer than cabbage.

The digestion of cabbage was studied and comparisons made of the gastric response to the raw and boiled forms of this vegetable. Even subjects who showed slow evacuation on most other types of foods had no trouble in evacuating cabbage. Thus a subject who required  $3\frac{1}{2}$  hours for either baked beans or cauliflower required only  $1\frac{1}{4}$  hours for raw cabbage (see fig. 23). Another subject who retained stewed veal  $3\frac{1}{2}$  hours, retained cabbage but  $1\frac{1}{4}$  hours (see fig. 18). Boiled cabbage required in one case the same period of digestion as the raw, in another case, however, 15 minutes longer. Boiling does not seem to increase the readiness with which foods of this character are handled by the stomach. That cooking decreases the nutritive value through loss of protein, carbohydrate, salts and antiscorbutics is well known.

Cold slaw required an hour longer to leave the stomach than plain raw cabbage (fig. 18). This can only be due to the added ingredients. Sauerkraut with vinegar required for one subject a gastric digestion period of 2 hours as compared with  $1\frac{1}{4}$  hours for plain raw cabbage.

Lettuce was given to two subjects, comparisons being made of plain lettuce with lettuce, sugar and vinegar, and with lettuce, olive oil and vinegar. A subject of the rapid-emptying type (fig. 18) showed a gastric digestion time of  $1\frac{1}{4}$  hours for plain lettuce as compared with 2 hours for the sugar-vinegar preparation and  $1\frac{3}{4}$  hours when the leaves were treated with olive oil and vinegar. A subject of a different type (fig. 23) showed an evacuation time of  $2\frac{1}{4}$  hours for the plain lettuce and 3 hours when oil and vinegar were added. The lettuce alone therefore threw a lesser burden upon the stomach and lettuce belongs to the most easily evacuated class of foods in spite of its great bulk as compared with equal weights of many such foods. This is related to the fact that such lettuce leaves the stomach almost unchanged and free acidities are quickly developed in the stomach after eating lettuce due to the low acid-combining power of the leaves. The response of the stomach to lettuce is, as indicated by the charts, very similar to that of cabbage in the same individuals.



*Celery and spinach and onions.* Raw and creamed celery were compared on two different subjects (see figs. 10 and 25). In both cases the creamed celery left the stomach sooner than the raw and in one case the difference was marked. This in spite of the fact that while 100 grams of the creamed product were fed in each case only 84 and 69 grams respectively of the raw stalk were ingested. In considering the rather slow evacuation of raw celery its pronounced flavor, high cellulose content, and the fact that large amounts of it are not ordinarily eaten at one time, must be borne in mind.

Boiled spinach was given to three subjects (see figs. 1 and 6). It remained in the stomach from 2 to  $3\frac{1}{4}$  hours, which was distinctly longer than for most other foods of the vegetable class.

Stewed and fried onions were fed to four subjects. Only 25 grams of the fried onions were fed. The stewed onions remained in the stomach longer than other vegetables low in protein, such as cauliflower or asparagus but not longer than such high protein vegetables as beans and peas (see figs. 17 and 22). The fried onions required from  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours to leave the stomach. It seems clear that while the irritating character of the onion delays its passage somewhat this delay is not notable where moderate amounts only of onions are taken.

*Chayotes, cucumbers, squash and tomatoes.* Chayotes were creamed and in this form tested out on four individuals of the rapid-emptying type. Evacuation times varied from  $1\frac{1}{4}$  to 2 hours. The acid responses were relatively low (see figs. 8, 9, 12, 14 and 16). The chayote belongs, therefore, in a class with those vegetables which leave the stomach most rapidly.

Cucumbers were given to two subjects each receiving first the raw sliced fruit and then on a later day the same amount of sliced cucumber which had been salted over night, the view being prevalent that such treatment renders this vegetable more digestible. The salted cucumbers did in fact leave the stomachs of these men a quarter of an hour sooner than the unsalted (figs. 13 and 26). It does not seem likely that this slight difference in evacuation time can be of great significance. The cucumbers left the stomach somewhat less rapidly than boiled corn or beets but much more readily than a meat. These vegetables do not therefore appear to impose any special burden upon the stomach. No ill effects were noted in any case.

In Korea "cucumbers are the most favored vegetables, and at one meal one gets them prepared in three or four ways—cucumber soup, salted cucumbers, fresh sliced cucumber and cucumber water. From

a baby who is hardly able to walk, up to the old, gray-haired men, everybody eats cucumbers and preferably unpeeled" (6).

Boiled squash remained in the stomachs of two subjects of the rapid-emptying type for  $2\frac{1}{4}$  and  $2\frac{1}{2}$  hours respectively (figs. 14, 15 and 16). This was about the same time as required for boiled carrots or sugar corn and a little longer than for raw carrots or tomatoes.

Tomatoes were fed to four men. Two of these subjects were fed raw tomatoes and then a few days later an equal quantity of the boiled fruit. Again the boiling of a vegetable was found to retard evacuation in this case by  $\frac{1}{2}$  to  $\frac{3}{4}$  hour (figs. 27 and 28). Raw tomatoes, it will be noted, left the stomach rapidly even with subjects of the slow-emptying type, this rapidity of evacuation being quite striking as compared with most of the foods tested.

*Mushrooms.* Mushrooms were fed to four subjects in amounts of 50 to 100 grams. In one case these mushrooms required 15 minutes longer to leave the stomach than raw endives which in turn gave a response similar to that of peas. In the other cases no direct comparisons were made with other vegetables as the principal object was to test the influence of mushrooms on the digestibility of meats which results will be published in another connection. It is clear, however, that subjects of the rapid-emptying type may require no longer than  $2\frac{1}{4}$  hours to evacuate as large amounts of mushrooms as 100 grams and hence these fungi cannot be said to throw any unusual burdens upon the stomach.

*Rice, polished and unpolished, corn and barley.* This group of foods consists, of course, of cereal foods but inasmuch as they are frequently used as substitutes for certain of the common vegetables some comparative tests may be of interest in this connection.

Boiled barley was tried out in only two cases. A subject of the rapid-emptying type evacuated this food in  $1\frac{1}{2}$  hours while a subject of the slow-emptying type required 3 hours (fig. 30) or about the same time as for unpolished rice.

Canned sugar corn was fed to three subjects. The best comparison is given by figures 15 and 16, showing the corn to leave the stomach in about the same time as raw carrots or tomatoes but more rapidly than boiled carrots or boiled squash.

Boiled hominy required 2 hours in the case of a subject of the rapid-emptying type and thus would appear to leave the stomach almost as rapidly as sugar corn.

Boiled polished and unpolished rice was fed to four subjects all of the slow-emptying type. One comparison of the two on the same subject would indicate that the unpolished rice remains in the stomach for a distinctly shorter time or  $2\frac{1}{2}$  hours as compared with 4 hours for the polished rice. In another case, however (fig. 2), the polished rice was evacuated in a relatively short time. One man emptied unpolished rice in about the same time as boiled barley (fig. 30) while figure 31 illustrates the response of a subject who required but  $2\frac{1}{2}$  hours for unpolished rice. It appears that unpolished rice is readily handled by the average stomach and usually passes on into the intestine sooner than polished rice similarly cooked.

*Agar-agar.* Inasmuch as many of the vegetables tested contained cellulose as a chief constituent, and in spite of the slight action of the gastric juice upon such cellulose, they left the stomach rapidly, it was thought of interest to determine the response of the stomach to nearly pure cellulose or hemicellulose. For this purpose agar-agar soaked in water was chosen. A comparison was made of the agar-agar with boiled unpolished rice (fig. 31). While the rice required  $2\frac{1}{2}$  hours and developed an acidity of 50, the agar-agar required only  $1\frac{1}{2}$  hours showing an acid development of 60. This rapid evacuation of hemicellulose is believed to throw considerable light on the problem of the reasons for the typical response of the stomach to certain classes of vegetables.

*Microscopical.* The samples of gastric contents withdrawn for fractional analysis were also in most cases examined microscopically with a view to determining the degree of disintegration, the extent of starch digestion and other changes.

Most of the vegetables which were fed in the raw condition, although they left the stomach rapidly, showed slow disintegration of the cells and cellular masses. This resistance to gastric digestion was noted for raw carrots, celery, tomatoes, lettuce, cabbage and cucumbers. Boiled carrots and cabbage showed much more complete disintegration and left the stomach later. Creamed celery and chayotes were also rather slowly broken up. In most cases boiled vegetables exhibited a rapid cellular disintegration. This was noted, for example, with boiled white and sweet potatoes, turnips, squash, peas and dasheen. Somewhat less rapidly broken up were fried potatoes, spinach, string beans, oyster plant, parsnips, baked beans, asparagus, beets, sugar corn and cauliflower. Potato chips were resistant due to their fat content. With stewed onions the larger cells were soon broken but the small cells were more resistant.

The starchy vegetables such as the potatoes were found to be considerably affected by salivary digestion. The starch-iodine reaction frequently became very slight early in gastric digestion, the red dextrin color only being obtained. In other cases the starch reaction decreased markedly by the end of one hour or toward the end of digestion. Baked beans and sugar corn showed considerable starch digestion although some cells giving the blue iodine reaction could nearly always be found.

*The formol titration.* The formal titration for amino acid groups was carried out in every case. In general it may be said that these values were either low and fairly constant throughout digestion (as might be expected in the case of foods of low protein content) or the values began high and gradually decreased to a low value. The latter we believe represent in the main cases where the residuum at starting was high in digestion products which became gradually diluted although in some cases it was due to a high initial concentration of amino substances in the foods themselves. Nothing like the marked progressive development of amino acid nitrogen occurring in the case of meats, etc., was noted for vegetables.

#### SUMMARY AND CONCLUSIONS

A study was made of the response of the normal human stomach to thirty different kinds of vegetables prepared in different ways. One hundred and twenty-four experiments were made on twenty-five normal men. The evacuation times and acid responses of the stomach were determined and physical and chemical changes in the ingested food noted. Subjects were classified as belonging to the slow- and rapid-emptying types.

The average evacuation time for all subjects of the rapid-emptying type was 2 hours and for the slow-emptying type  $2\frac{1}{2}$  hours. The averages of the high points of the total acid curves were 70 and 77 respectively.

The response of the stomach to potatoes prepared in the following different ways was determined: whole boiled, creamed, mashed (with and without milk and butter), baked (with and without butter), potato salad, French, German and plain fried potatoes, and potato chips. All of these left the stomach in moderate time or  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours for rapid type individuals and 2 to  $3\frac{1}{2}$  hours for the slow type. Baked potatoes with butter required more than the average time but plain baked potatoes about the same time as boiled potatoes. The addition of

milk and butter to mashed potatoes had little effect nor did creamed potatoes or potato salad differ much from plain boiled potatoes as regards gastric response. Fried potatoes left the stomach as rapidly as potatoes prepared in other ways. French fried potatoes a few minutes longer than German or plain fried potatoes. Potato chips were handled very rapidly by the stomach considering their high solid and fat content.

Sweet potatoes whether boiled, baked or fried remained longer in the stomach than white potatoes cooked in the same ways. Dasheens, whether boiled, baked or fried required a longer period of gastric digestion than white potatoes but no longer than sweet potatoes. Cassava roots were fed boiled and baked and found to require a little longer than white potatoes similarly prepared. Boiled oyster plant required about the same period of gastric digestion as white potatoes.

Boiled red beets left the stomach rapidly, i.e., in from 1 to 2 hours. After being pickled in vinegar they left still more rapidly, the vinegar evidently hastening the evacuation. Raw carrots also left the stomach rapidly (in from  $1\frac{1}{2}$  to 2 hours). Boiled carrots took distinctly longer ( $2\frac{1}{4}$  to  $2\frac{1}{2}$  hours). Boiled parsnips required distinctly longer than beets or carrots. Boiled turnips left the stomach in  $1\frac{1}{2}$  hours. Radishes in amounts of 50 grams left the stomach very rapidly (in from  $1\frac{1}{4}$  to  $1\frac{1}{2}$  hours).

Baked beans, as might be expected from their high protein content, remained somewhat longer in the stomach than most other kinds of vegetables and developed higher combined acidities. String beans, on the other hand, left the stomach rapidly resembling more closely the green vegetables in stimulatory power upon the stomach. Boiled peas required  $1\frac{1}{4}$  hours in each of two cases or less time than baked beans. Boiled lentils appeared to require a little longer than peas while raw endives resembled peas as far as evacuation times were concerned.

Asparagus left the stomach rapidly. Boiled cauliflower required only a few minutes longer in one case, but in a subject of the slow type showed delayed evacuation.

Cabbage whether raw or cooked left the stomach rapidly even in subjects of the slow-emptying type. Boiled cabbage was not handled more readily than the raw. Cold slaw and sauerkraut required distinctly longer to leave the stomach than plain cabbage.

Lettuce in spite of its bulky character was found to be one of the most easily evacuated of foods, the response to this food resembling

that to cabbage. The addition of sugar and vinegar or oil and vinegar delayed evacuation.

Celery in the raw state is evacuated more slowly than cabbage or lettuce but creamed celery caused less difficulty. Spinach remained in the stomach distinctly longer than most vegetables.

Onions stewed and fried showed slower evacuation than other vegetables low in protein, but not longer than high protein vegetables, when fed in moderate amounts.

Creamed chayotes left the stomach rapidly (in from  $1\frac{1}{2}$  to 2 hours), cucumbers did not impose any special burden on the stomach, whether salted or unsalted. The unsalted required but a few minutes longer than the salted cucumbers.

Boiled squash required  $2\frac{1}{4}$  to  $2\frac{1}{2}$  hours or about the same time as boiled carrots or sugar corn.

Raw tomatoes left the stomach very rapidly in all cases. Boiled tomatoes required a longer time.

Mushrooms require only a moderate period of gastric digestion— $2\frac{1}{4}$  hours with a subject of the rapid type.

Stewed corn left the stomach almost as rapidly as raw carrots or tomatoes and boiled hominy required but little longer.

Boiled unpolished rice and boiled barley were readily handled by the stomach and usually passed into the intestine sooner than boiled polished rice.

Raw agar-agar left the stomach in  $1\frac{1}{2}$  hours as compared with  $2\frac{1}{2}$  hours for boiled unpolished rice. Hemicelluloses appear therefore to leave the stomach very rapidly and show a certain analogy with vegetables such as cabbage and lettuce.

In general raw vegetables low in protein, as carrots, celery, tomatoes, cabbage, lettuce and cucumbers, leave the stomach rapidly, develop moderately high free acidities but little combined acidity and leave the stomach without great change. Boiled vegetables show much more rapid and complete disintegration. Vegetables high in starch such as potatoes show very considerable starch digestion before leaving the stomach. In certain cases hardly any starch reaction could be obtained toward the end of digestion.

The authors desire to thank, for their coöperation, the many medical students who sacrificed time and convenience to serve as subjects of these experiments.

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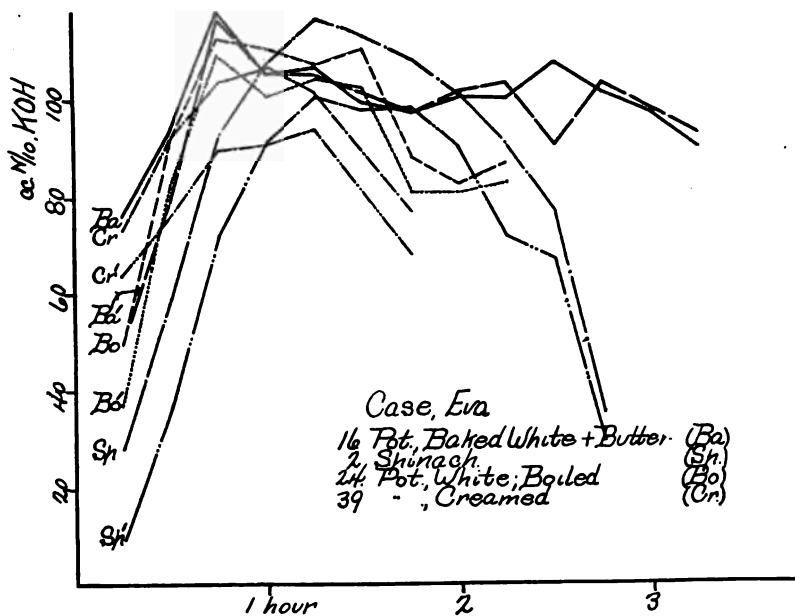


FIG. 1

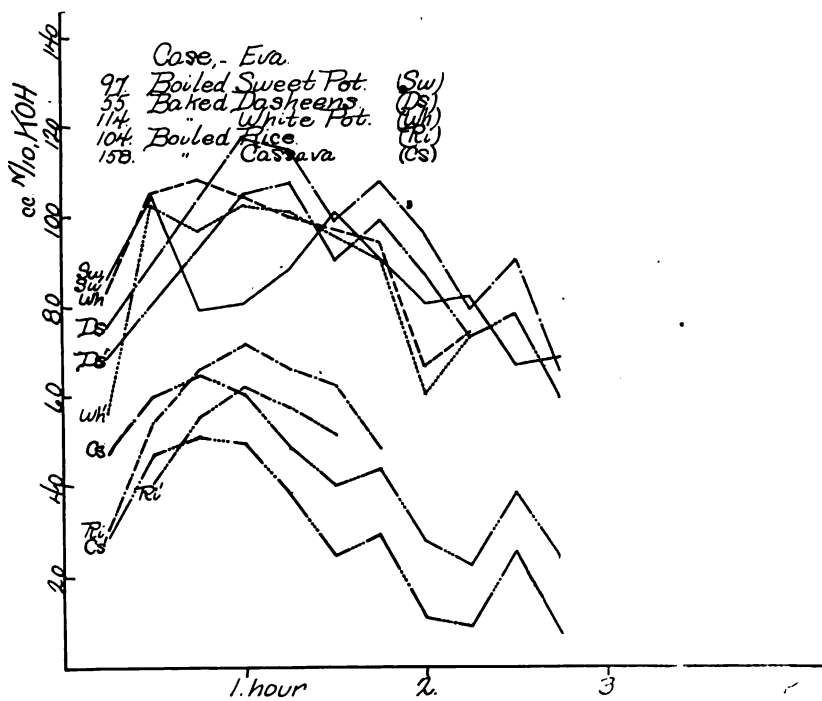


FIG. 2



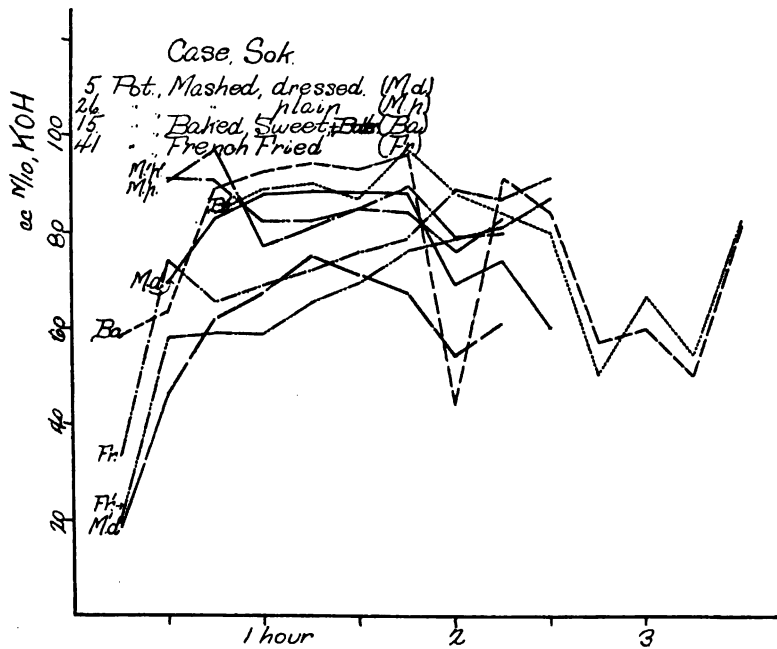


FIG. 3

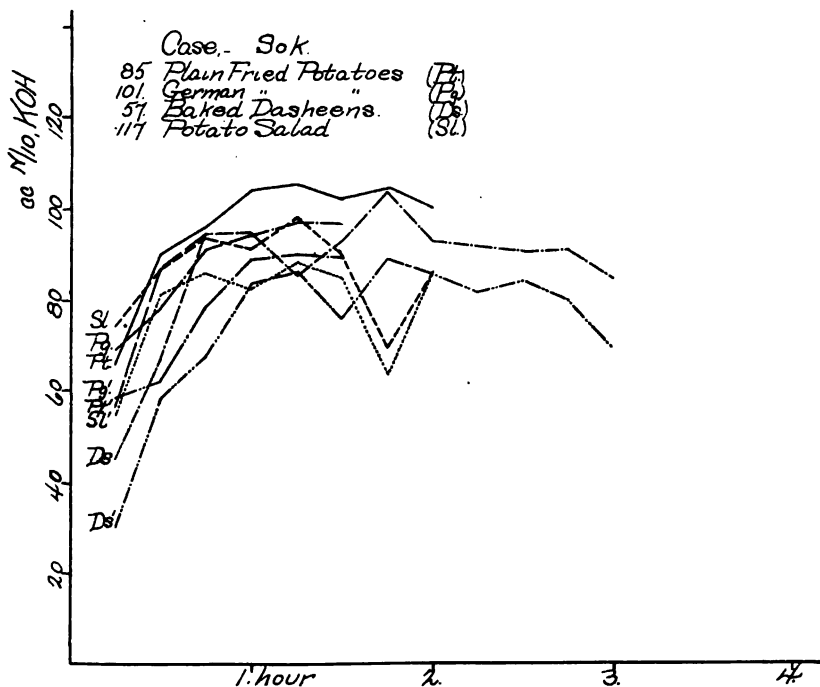


FIG. 4

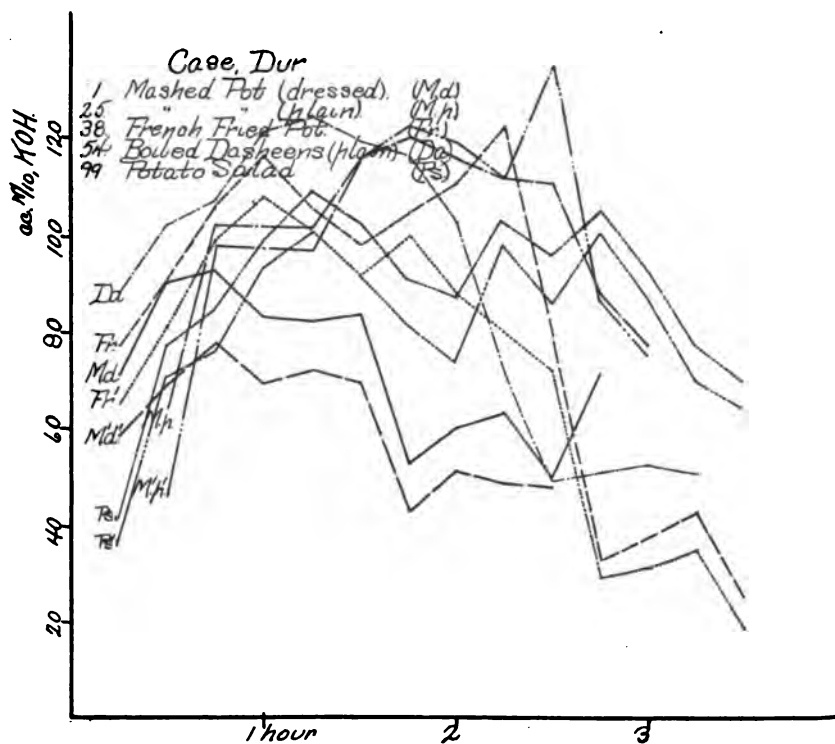


FIG. 5

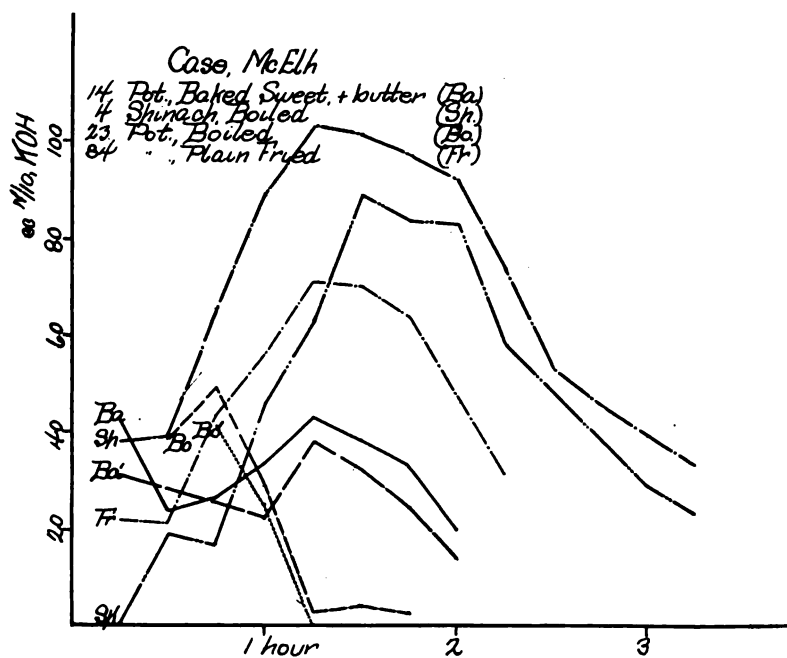
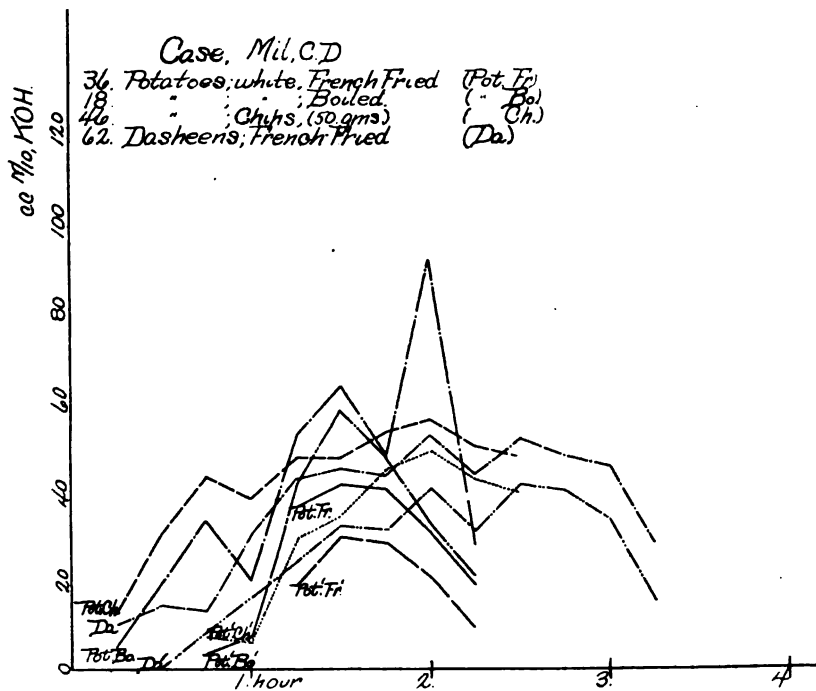
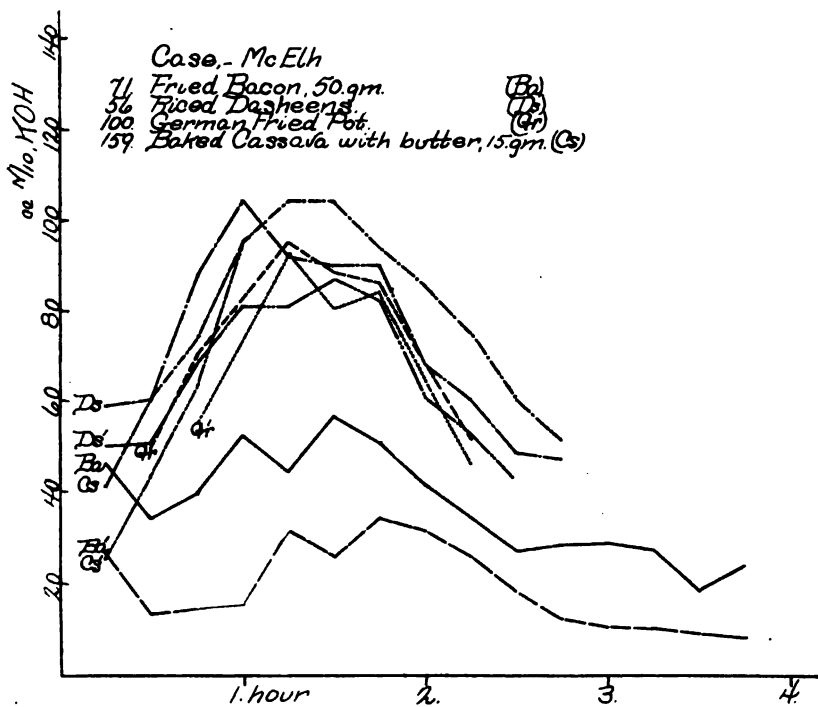


FIG. 6



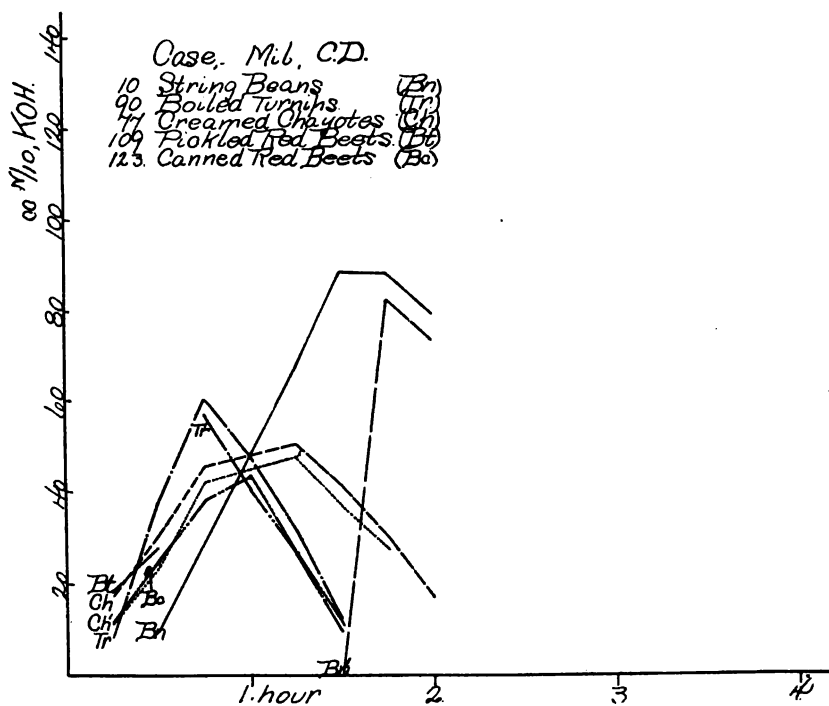


FIG. 9

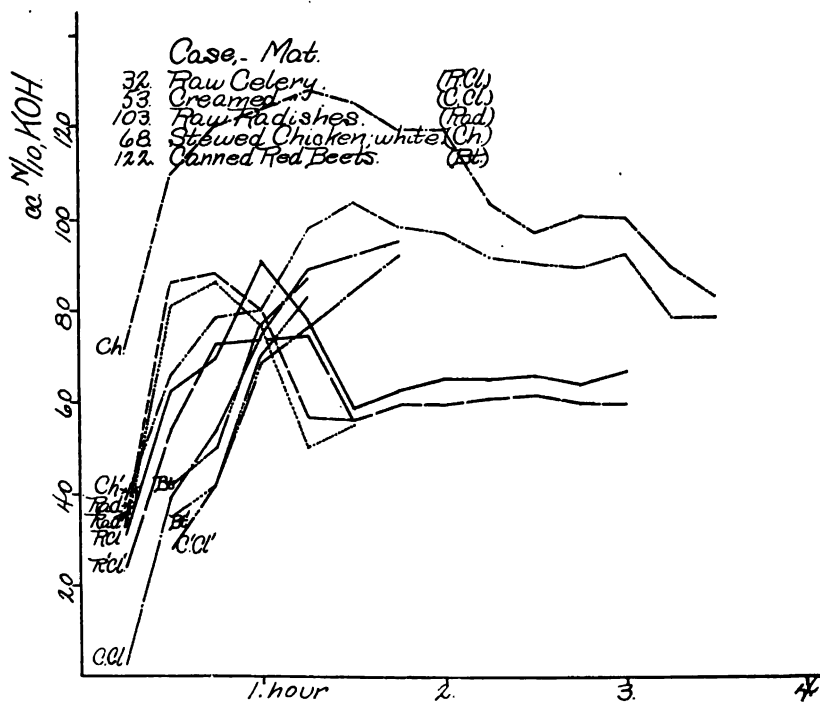


FIG. 10

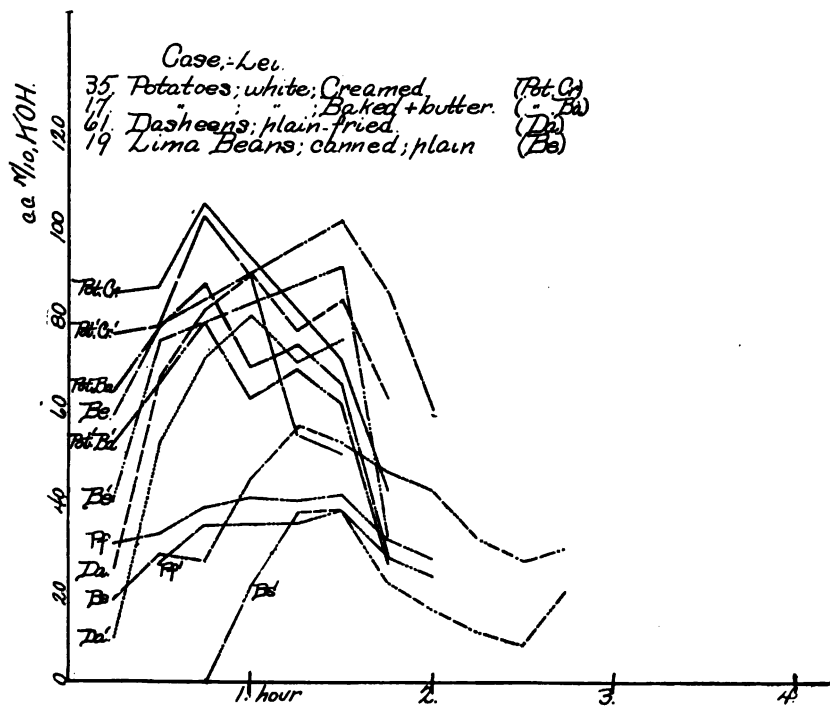


FIG. 11

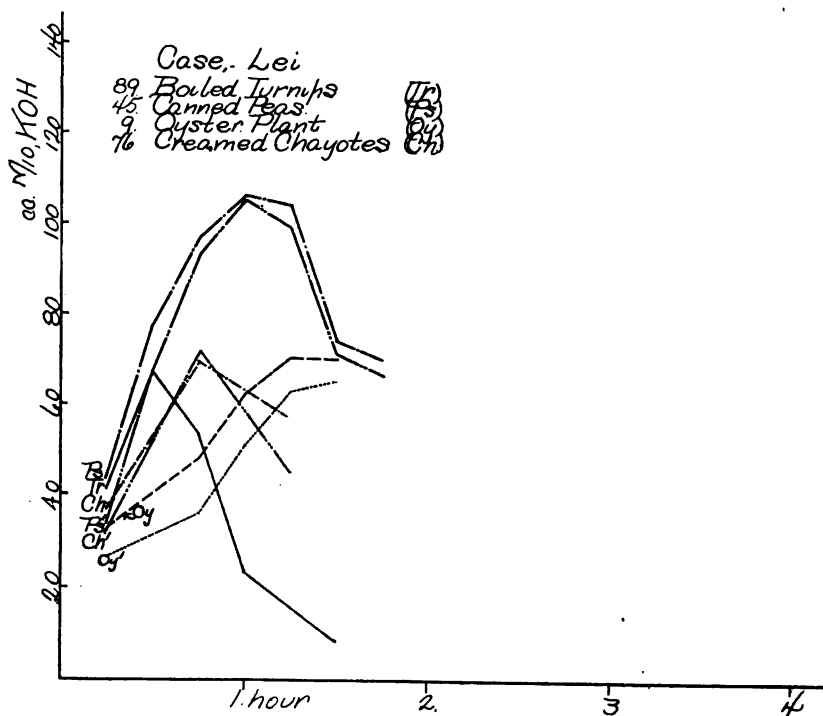


FIG. 12

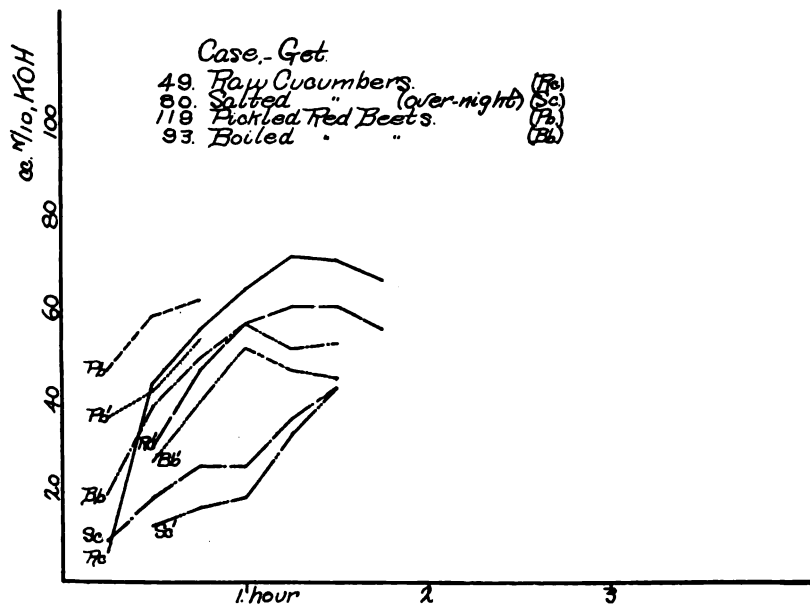


FIG. 13

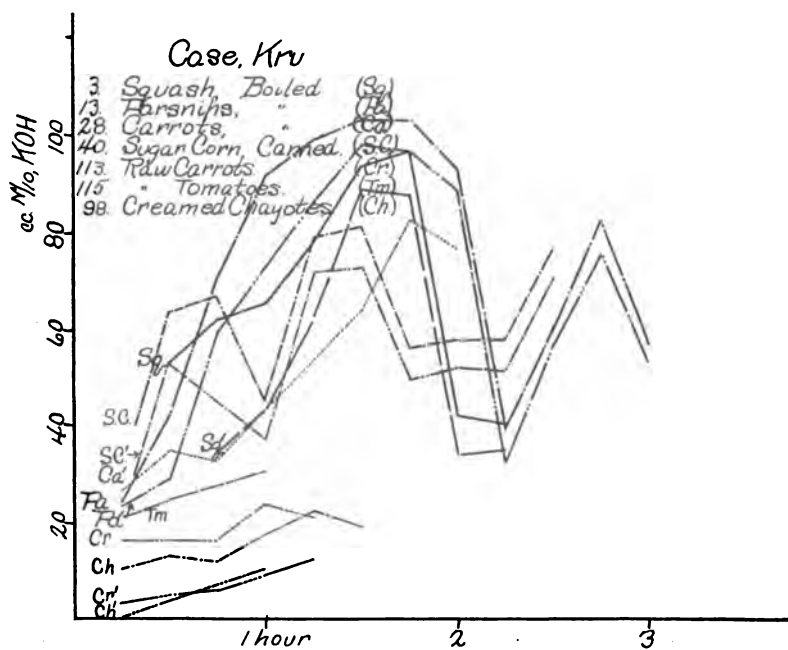


FIG. 14

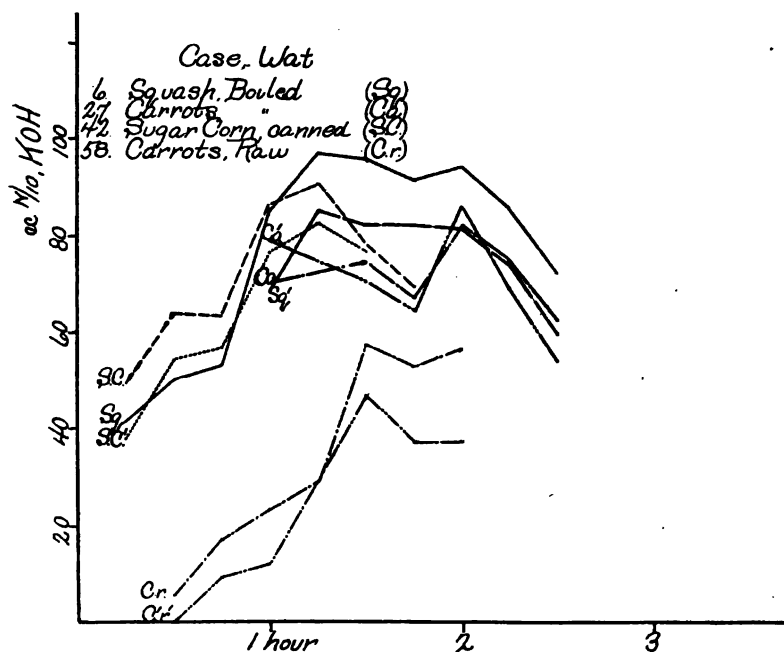


FIG. 15

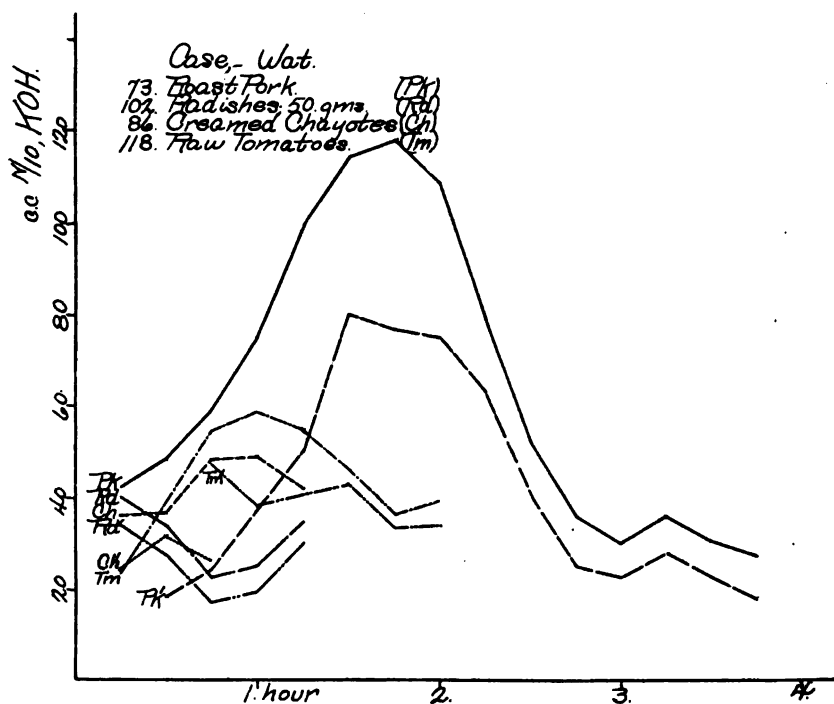


FIG. 16

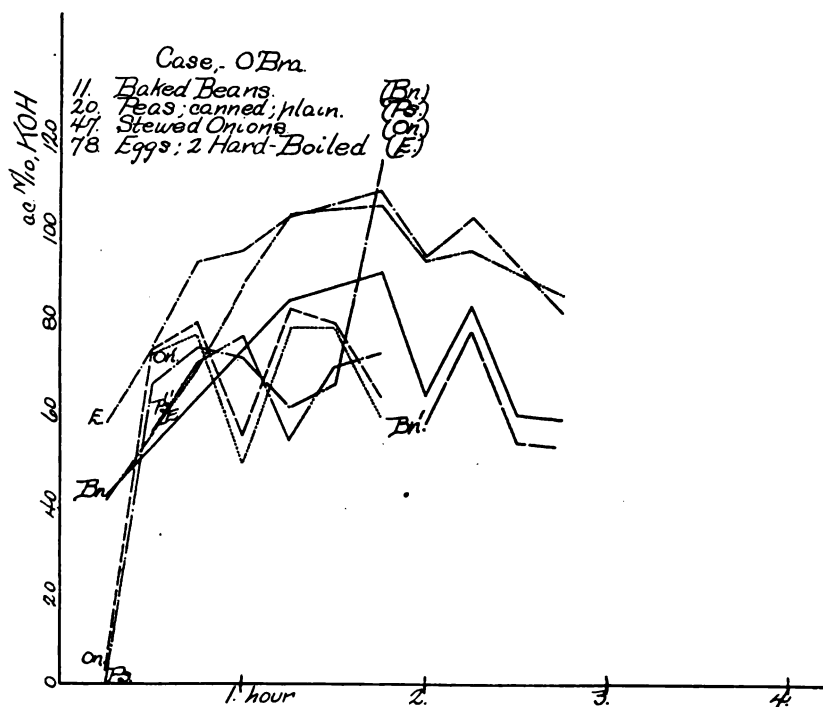


FIG. 17

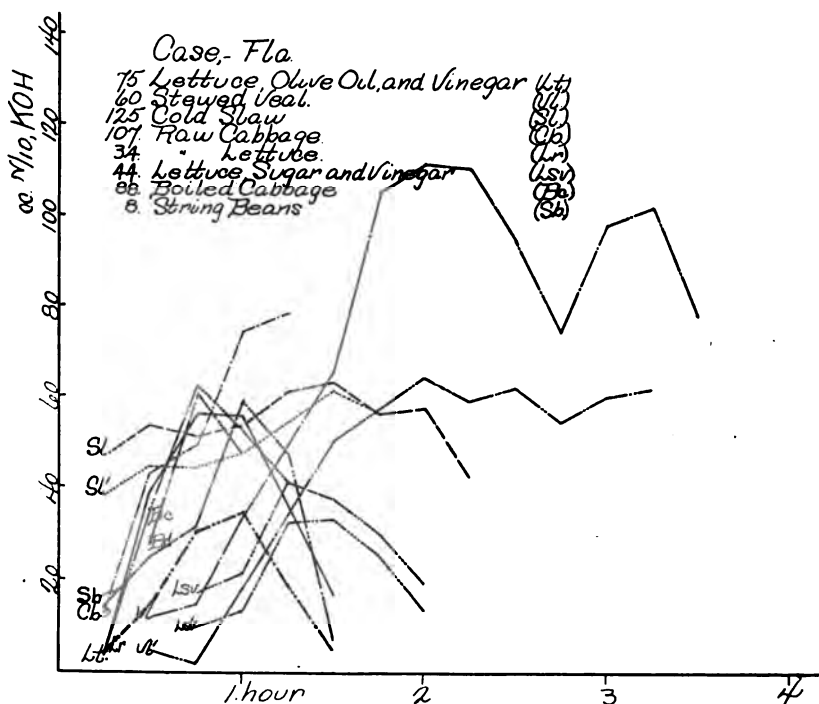


FIG. 18



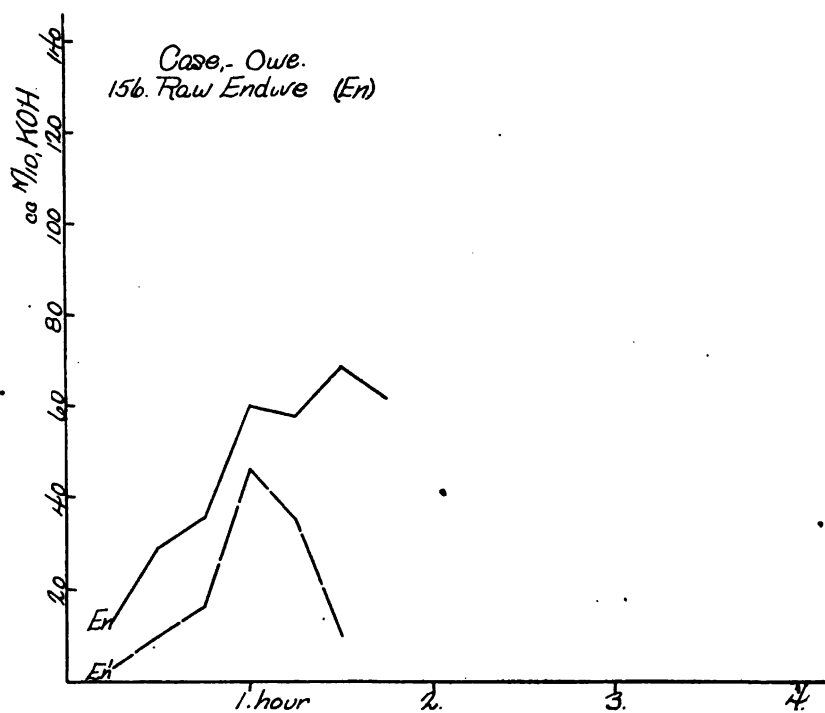


FIG. 19

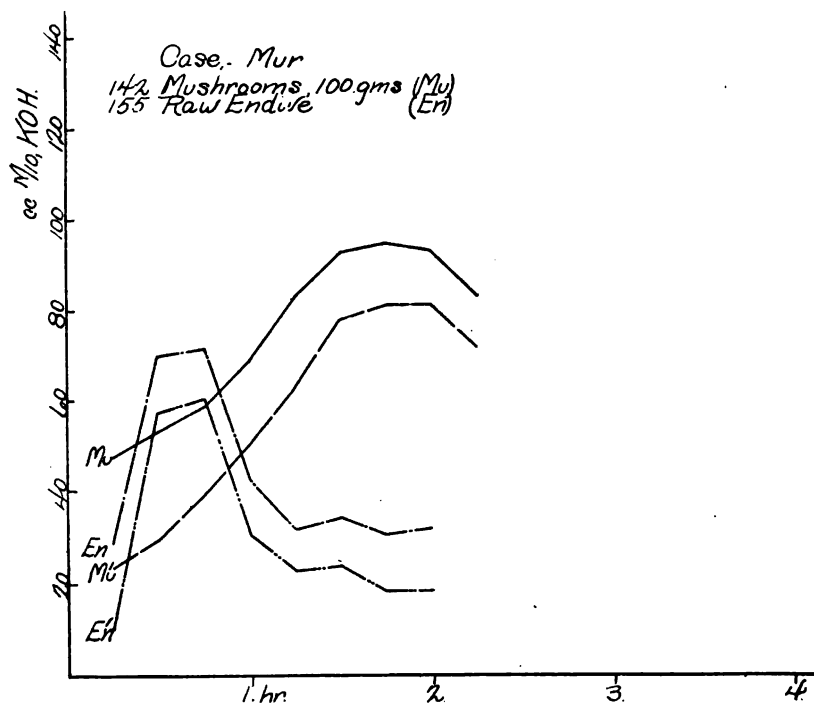


FIG. 20

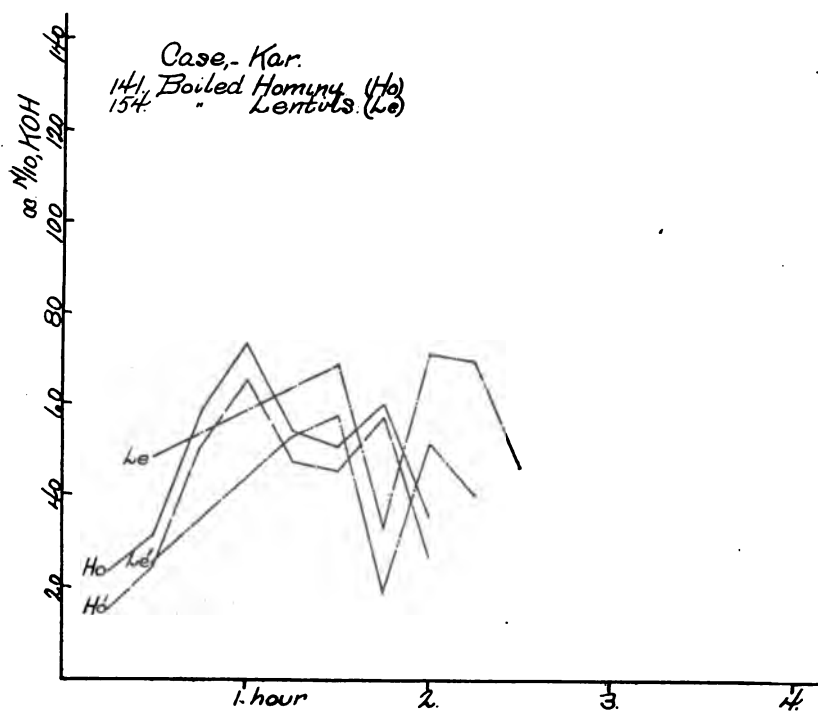


FIG. 21

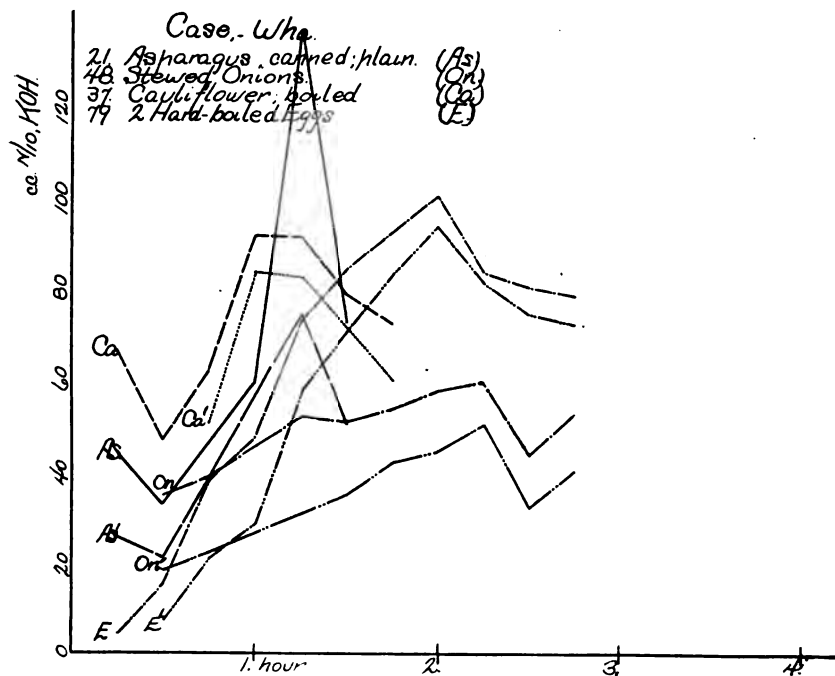


FIG. 22

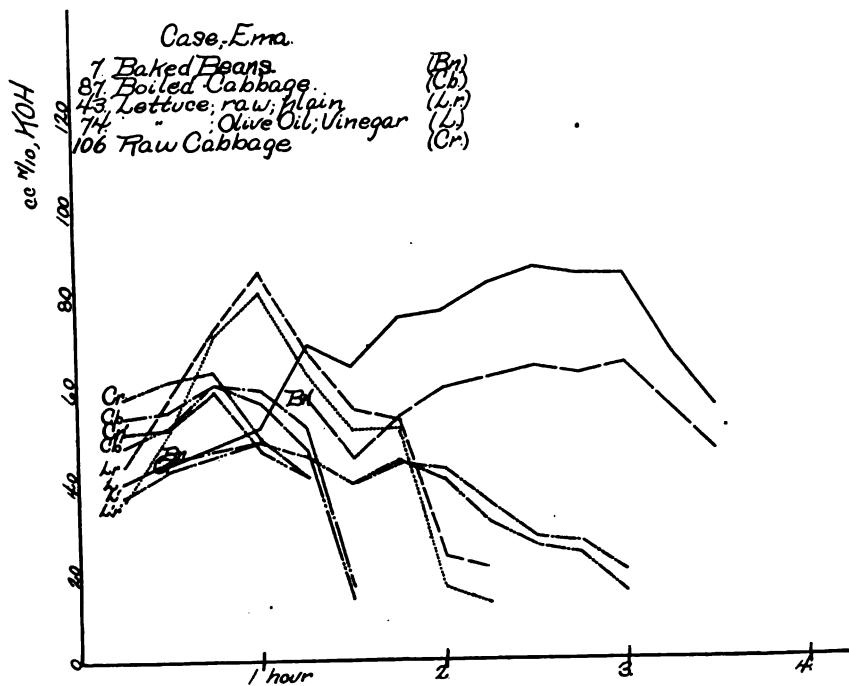


FIG. 23

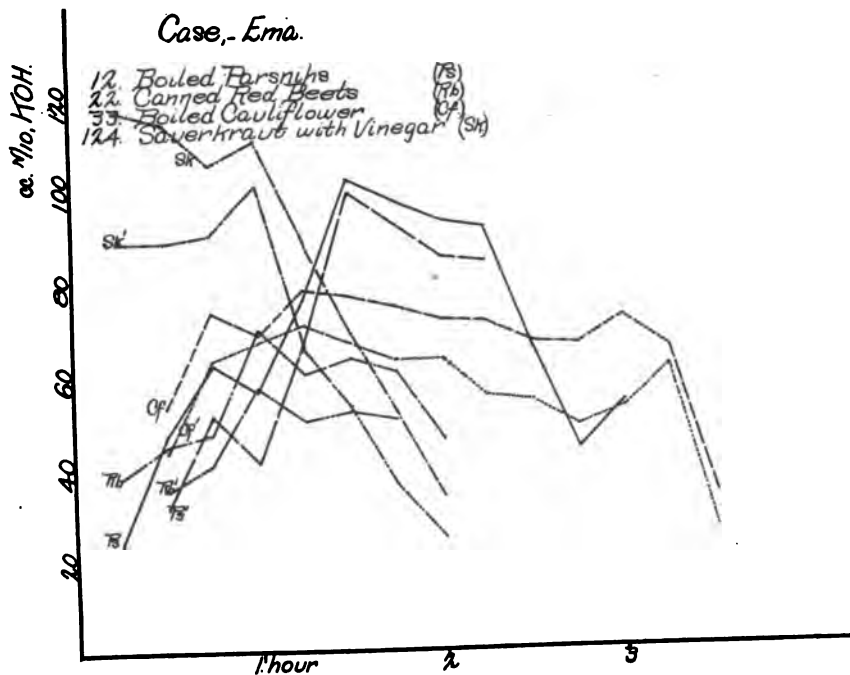


FIG. 24

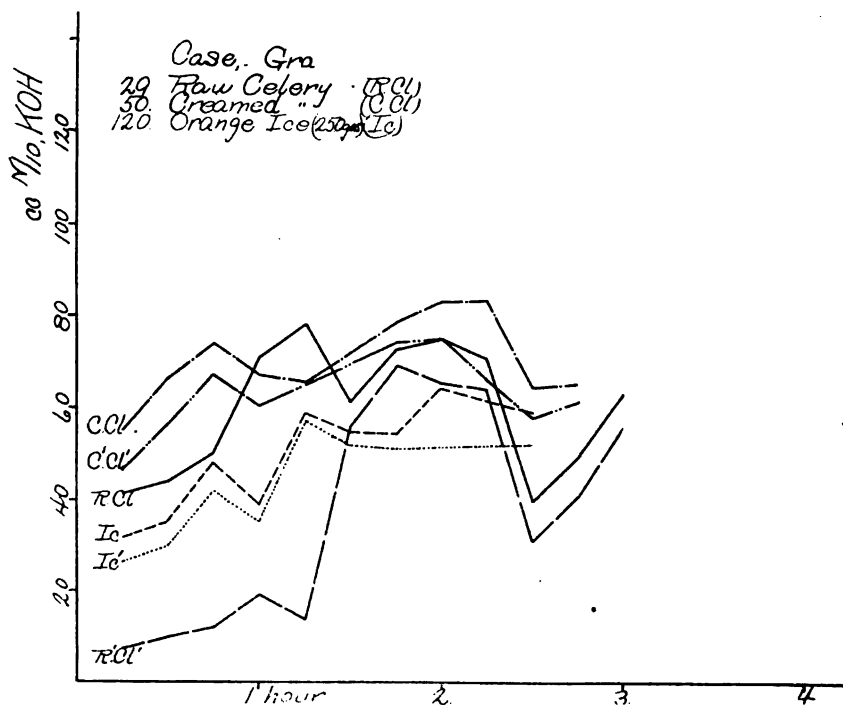


FIG. 25

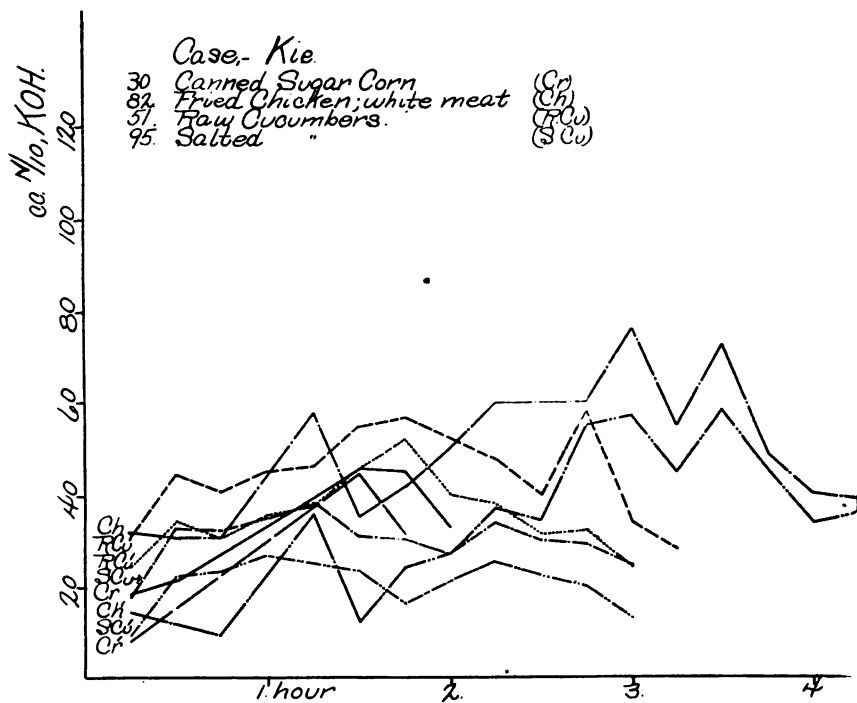


FIG. 26

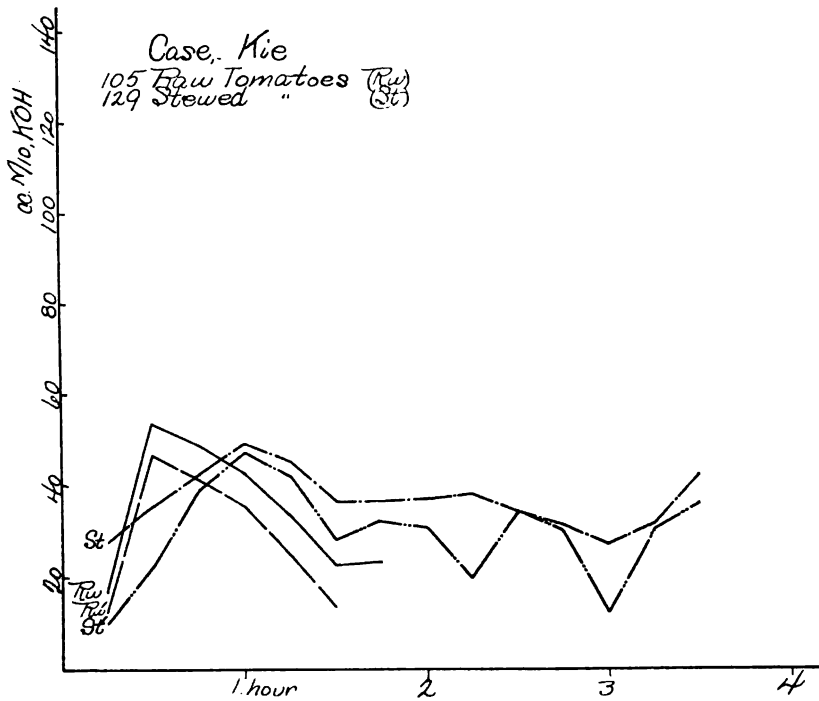


FIG. 27

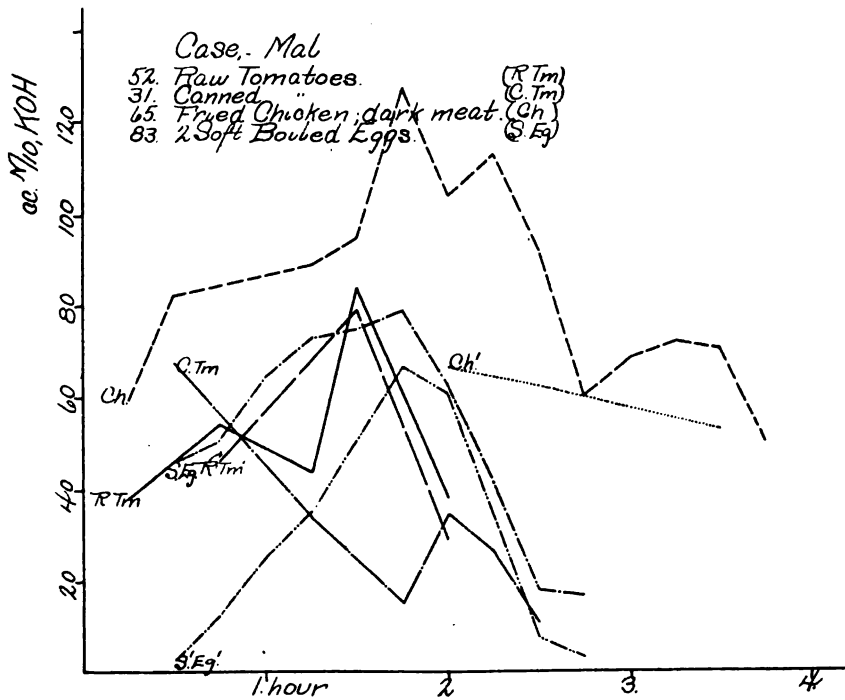


FIG. 28

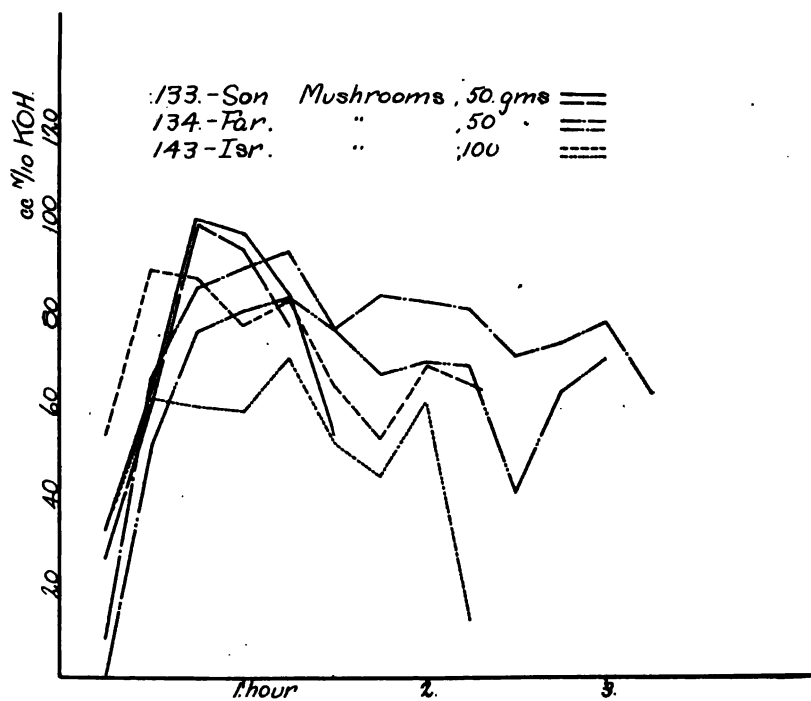


FIG. 29

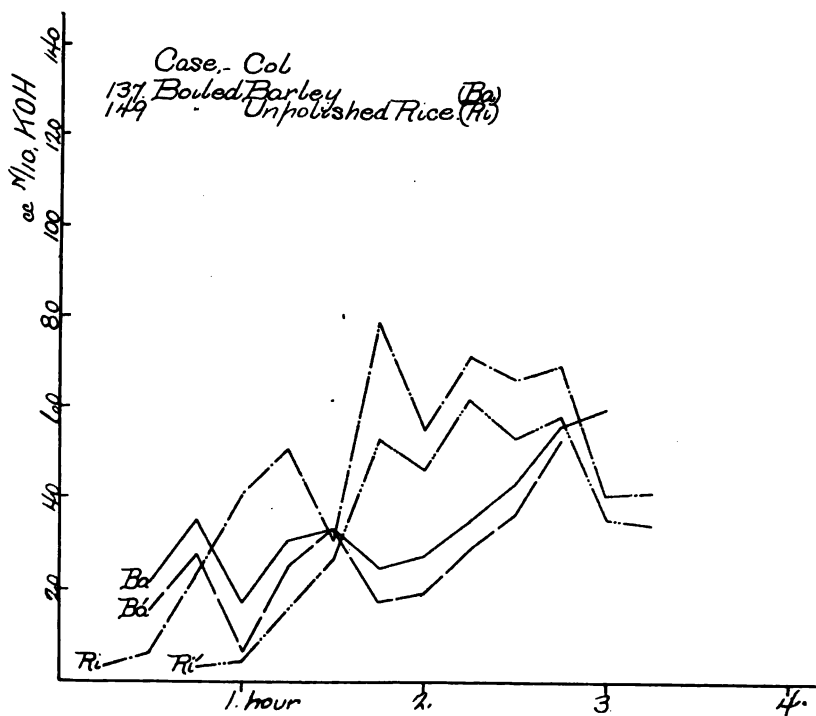


FIG. 30

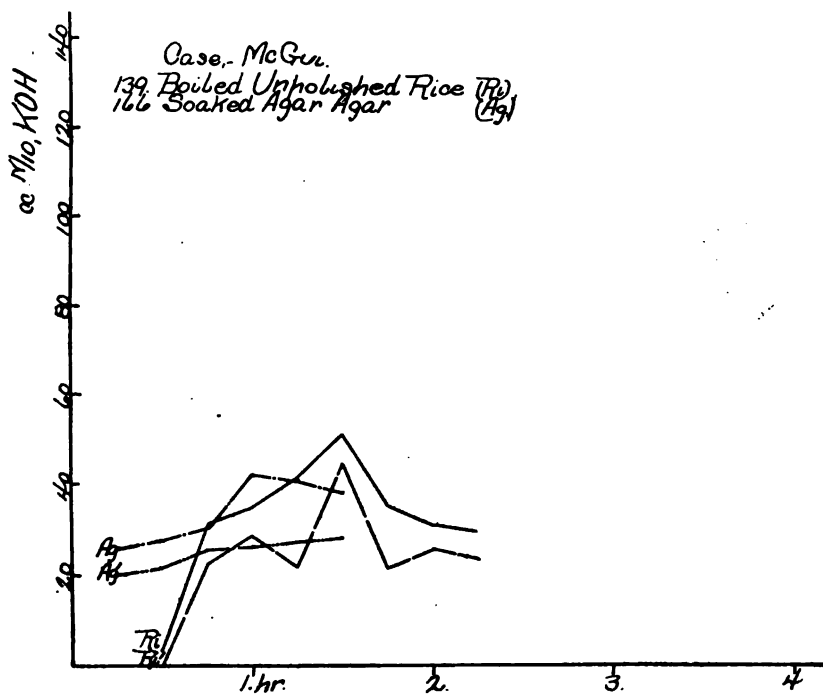


FIG. 31

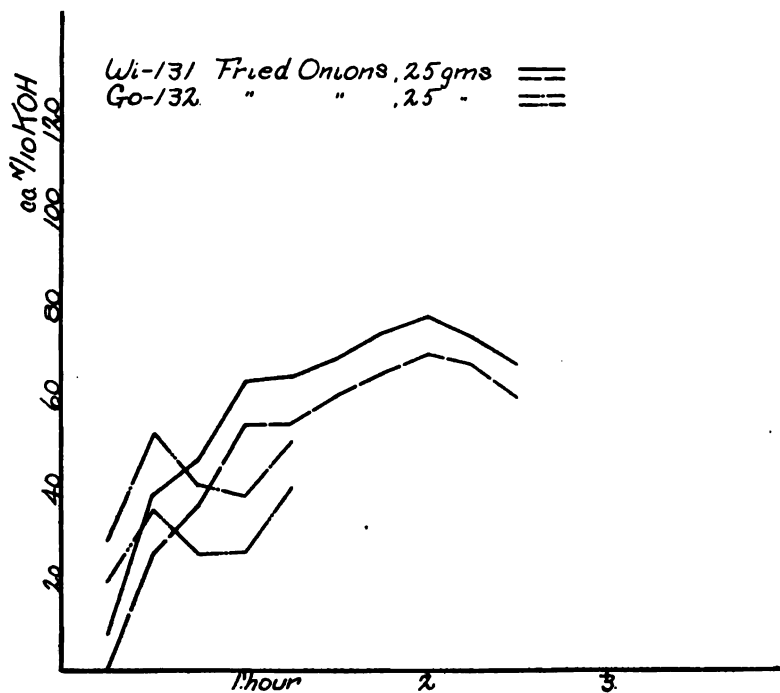


FIG. 32

365

## FURTHER OBSERVATIONS ON THE RELATION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPER- GLYCEMIAS (ETHER AND ASPHYXIA)

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The question whether the epinephrin output of the adrenals is essentially concerned in the production of certain experimental hyperglycemias has been much debated. Two main ways of approaching the question are theoretically open: *a*, the estimation of the output of epinephrin under the action of the factors inducing the experimental hyperglycemia, in order to show whether this can be sufficiently great to permit the introduction into the blood of the necessary excess of epinephrin as determined by artificial injection; *b*, study of the blood sugar content in the absence of the adrenals or after interference with their epinephrin secretion, when the conditions which cause hyperglycemia with intact adrenals are induced. The first method of approach might seem to be the more direct, but it has been little used. Such results as have been obtained on the rate of output of epinephrin in ether narcosis and asphyxia are quite unfavorable to the view that epinephrin plays any sensible rôle in the production of the hyperglycemia.

Of the numerous researches made by the second method many, unfortunately, however interesting in other relations, throw no light upon the question at issue because essential conditions were not fulfilled. The most important of these seem to be the following:

1. The glycogen store must be adequate to permit of a decided hyperglycemia. Negative results cannot otherwise be accepted. The only sure way is to estimate the liver glycogen.

2. Only animals which have completely recovered from the effects of the operation practised to eliminate the adrenal epinephrin output should be used. Cats as employed by us, after removal of one adrenal and section of the nerves of the other, and rabbits which have survived double adrenalectomy, fulfil this condition.



3. The question of the rôle of the adrenals should not in the present state of our knowledge be complicated by unnecessary interference with the nerve supply of the liver. For example, negative results after section of both splanchnics cannot be used to determine the question of the indispensability of the adrenals. In our experiments on ether, asphyxia (1) and piqué hyperglycemia (2) only one splanchnic was cut in the cats and neither splanchnic in the rabbits.

We believe it is inadvisable to complicate the question whether the adrenals are essential for the development of experimental hyperglycemias with speculations on the possible output of epinephrin from the diffuse chromaffine tissue. There is no evidence as to the possible magnitude of the output of epinephrin, if there is any output, from these scattered chromaffine cells and no obvious way of investigating the matter.<sup>1</sup> Our own experiments have been concerned solely with the adrenals. Whether they are essential for the hyperglycemias studied is a question which can be definitely settled. We believe it has been definitely decided in the negative, and that the so-called "adrenalin hypothesis" should be abandoned, at least in the case of these forms of hyperglycemia.

Recently, however, Keeton and Ross (3) have published a paper on the mechanism of ether hyperglycemia in dogs, in which they make the point that a short etherization is associated with some hyperglycemia even when both splanchnic nerves have been cut, but that this does not persist, under continuous insufflation of ether, as it does in the case of normal dogs. Incidentally they raise the question whether the etherization in our experiments was continued long enough. They think it was not, and that we might have obtained a different result with a longer period of etherization. For this reason they do not consider that we have demonstrated conclusively that ether hyperglycemia can occur in the absence of the epinephrin output of the adrenals. Section of both splanchnics introduces the complication that the innervation of the liver is greatly interfered with, whereas our object was to interfere only with the adrenals, and in this regard

<sup>1</sup> If the diffuse chromaffine tissue is under the control of nerves in respect of any output of epinephrin, as is to be supposed since it represents sympathetic nerve cells, the output could be greatly interfered with, it may be assumed, by section of sympathetic fibers, including the splanchnics, or by appropriate spinal cord section. In animals which have survived in good health some of the operations practised by us, it seems probable that a large part of the epinephrin output, if any exists, of the diffuse chromaffine tissue was suppressed as well as that of the adrenals.

our experiments are not really comparable with those of Keeton and Ross. Nevertheless, as courteous criticism is always helpful and as, of course, it might be possible that a longer period of etherization should develop a difference between normal animals and those in which the adrenal epinephrin output has been interfered with, we have made some experiments in which the blood sugar was determined at the end of a short period (20 minutes) and again at the end of a much longer period of etherization (80 to 90 minutes). The experiment was wound up with a period of asphyxia and another blood sample collected, since asphyxia is one of the surest methods of producing hyperglycemia, and in the event of a negative result with ether the asphyxia sample serves as a control to show whether the nutritive condition of the animal, especially the glycogen store, was compatible with a well marked hyperglycemia. In addition, the glycogen content of the liver was estimated.

Before proceeding to these experiments, however, it seems necessary to explain what we aimed at in our previous experiments. Keeton and Ross state that "of four of their (Stewart and Rogoff's) experiments, only two show a gain (0.087 to 0.151 and 0.142 to 0.176) that does not fall within the limit of experimental error. The other two (0.200 to 0.233 and 0.092 to 0.098) are not significant." This criticism is based on a misapprehension. We made a number of blood sugar experiments on each cat at different dates. In some instances the results were negative in one of the experiments, while on another day, perhaps a week or more thereafter, the animal meanwhile being specially fed, the results were positive, doubtless owing to the difference in the nutritional state (glycogen content of liver), a condition which is known to be of the highest importance for the production of such experimental hyperglycemias. Precisely similar results are obtained in normal animals.

In the new experiments rabbits which had survived the removal of both adrenals were employed, and here of course there was no question of any residual liberation of epinephrin from the adrenals. The animals had perfectly recovered from the primary operations, and for 8 to 11 days before the blood sugar tests were placed on a diet of carrots in addition to the routine diet of oats and hay, and sugar was added to the drinking water twice a day. The glycogen content of the liver was estimated at the end of the experiment. The results, as will be seen from the following protocols, were entirely confirmatory of our results on cats.

*Condensed protocol. Rabbit 409*

September 19, 1919. Excised right adrenal.

October 21, 1919. Excised left adrenal. Weight of rabbit 2.51 kgm.

November 17 to November 25, 1919. Carrots in addition to usual diet (oats and hay) and cane sugar added to drinking water, twice daily.

November 25, 1919. Condition excellent. Weight 2.67 kgm.

9.30 a.m. 2 cc. blood (from ear vein) contained 0.13 per cent dextrose.

9.40 a.m. Started light etherization.

10.05 a.m. 2 cc. blood (from ear vein) contained 0.16 per cent dextrose.

Continued light etherization for an hour longer.

11.10 a.m. 2 cc. blood (from ear vein) contained 0.27 per cent dextrose. Now discontinued etherization.

11.25 a.m. Started asphyxia and continued it intermittently till

11.45 a.m. 2 cc. blood (from ear vein) contained 0.37 per cent dextrose.

The liver, excised and hydrolyzed at once, contained 3.13 per cent of glycogen. The liver weighed 40.3 grams.

Of course, the glycogen content at the beginning of the experiment would be decidedly greater than that estimated at the end.

*Condensed Protocol. Rabbit 410*

September 19, 1919. Excised right adrenal.

October 21, 1919. Excised left adrenal. Weight of rabbit 3.18 kgm.

November 17 to November 28, 1919. Carrots in addition to usual diet (oats and hay) and cane sugar added to drinking water, twice daily.

November 28, 1919. Condition excellent. Weight 3.25 kgm.

9.30 a.m. 2 cc. blood (from ear vein) contained 0.11 per cent dextrose.

9.40 a.m. Started etherization (to surgical anesthesia).

10.00 a.m. 2 cc. blood (from ear vein) contained 0.27 per cent dextrose. Continued etherization (lightly) for an hour longer.

11.05 a.m. 2 cc. blood (from ear vein) contained 0.41 per cent dextrose. Now discontinued etherization.

11.15 a.m. Started asphyxia: after 7 minutes of asphyxia (with a towel over nose) applied off and on (being guided by the slowing of the heart rate) the circulation became poor and the respirations shallow and slow. Artificial respiration was started and an attempt made to obtain blood from the femoral vein, but failed, owing to the poor blood flow. The chest was opened and 2 cc. of blood were obtained from the right heart. The heart was beating feebly and the blood was dark. This specimen contained 0.53 per cent dextrose.

The liver was excised 4 minutes after the last specimen was obtained and hydrolyzed at once. It contained 2.5 per cent of glycogen at the end of the experiment. At the beginning the glycogen content must have been greater. The surplus sugar in the blood at the end would alone correspond to an additional amount of 0.7 per cent glycogen in the liver.

Our results on asphyxial hyperglycemia (1), (2) both in cats after interference with the adrenal epinephrin output and in rabbits after

removal of both adrenals are quite as conclusive as those on ether hyperglycemia. Where the liver was well filled with glycogen a marked hyperglycemia was invariably obtained. What interpretation can possibly be placed upon such results except that the adrenal epinephrin is not essential to the production of these hyperglycemias?

Yet a quite recent writer, Yamakami (4), seems to think that direct evidence of this kind can be set aside because he has, as he supposes, shown that asphyxial blood, obtained from one normal rabbit and injected into another normal rabbit causes a rise in the sugar content of the blood. Even if the results which he quotes be accepted as proving a distinct augmentation in the blood sugar of the recipient rabbit, in addition to any increase due to the sugar actually present in the injected asphyxial blood (and not more than half of the experiments reproduced in his table could be considered positive), they are irrelevant to the question of the rôle of the adrenals in asphyxial hyperglycemia. They do not warrant "the hypothesis that adrenalin in the asphyxial blood is responsible because we do not know at present any other substance than adrenalin in the blood which can give rise to the enhanced sugar content." He endeavors to exclude changes in carbon dioxide content and possible changes in H-ion concentration in the asphyxial blood as factors. It would seem a more crucial test to estimate the epinephrin in the blood injected, since it is universally acknowledged that a certain amount of epinephrin will cause hyperglycemia. Underhill (5) showed that very large quantities of adrenalin introduced by continuous intravenous infusion into non-anesthetised rabbits (as much as 333 cc. of a 1:250,000 solution) did not cause glycosuria. It must be noted that the heart blood was taken from nearly dead, or in half the cases, from actually dead rabbits. With the extreme slowing of the blood flow in the inferior cava the concentration of epinephrin in the heart blood, so long as the adrenal epinephrin output was not affected, would tend to rise. The Japanese author seems to have had some suspicion that the adrenalin hypothesis might not have much to support it, for he continues "of course, we cannot venture to claim that hyperadrenalinemia was proved by our experiments to exist in asphyxia." Curiously enough he states early in the paper that "in order to solve the problem whether the adrenals are involved in the asphyxial hyperglycemia it seems to be the wisest method to study this hyperglycemia in animals whose adrenals were removed entirely," and he suggests rabbits which have survived double adrenalectomy as the most suitable. But he makes no mention of our

observations on piqûre and asphyxial hyperglycemia in such rabbits, which demonstrate that the adrenals are unnecessary. Nor does he utilize in his own observations what would be a really crucial experiment, the injection of asphyxial blood from an adrenalectomized rabbit into a normal rabbit, and into another adrenalectomized rabbit. He seems to confuse our method of collecting adrenal vein blood with the method of collecting specimens for blood sugar estimations. We did not of course anesthetize the animals, perform a laparotomy, etc., to obtain blood specimens when we were determining whether asphyxia could cause hyperglycemia in the absence of epinephrin discharge from the adrenals.

Kellaway (6) has recently verified our conclusion that the adrenal epinephrin output is not essential to the production of the hyperglycemia induced by asphyxia. He states that we have "denied that the suprarenals play any part in producing the hyperglycemia." We have not as a matter of fact put our conclusion in so absolute a form because, although our observations do not reveal any essential difference between normal animals and animals with the epinephrin output interfered with, in the degree of the hyperglycemia or the ease and certainty with which it is induced, it would be very difficult in experiments of this type to bring out clearly a small quantitative difference if such existed. Kellaway's blood sugar results in normal cats and in cats after interference with the adrenal epinephrin output are very much like our own, despite the fact that in his observations both splanchnics were divided and the innervation of the liver crippled.

His contention that increased adrenalin output is a factor in the hyperglycemia is not supported by his results so far as we can see. In most of his experiments he gets a good hyperglycemia with anoxemia (or asphyxia) after interference with the epinephrin output, exactly as we found. He has not estimated the liver glycogen in any of his animals nor does he indicate anywhere that he realizes its importance. He seems to assume that "the value of the threshold for anoxemia as regards hyperglycemia" can be fixed once for all for a given animal and that if breathing a certain percentage of oxygen causes hyperglycemia days or weeks before section of the splanchnics or removal of the adrenals, and fails to cause it after those operations, the difference must necessarily be due to the absence of epinephrin. It is impossible to accept the conclusion that because in one cat (exper. 14) "there was a very close correspondence between the production of a pupillary paradox and a rise in blood sugar" before section of the splanchnics and removal of

the adrenals, and because both reactions were absent or greatly reduced after these operations "it seems evident that in this cat the blood sugar effects were largely due to adrenalin." The failure to obtain hyperglycemia at the end of the experiment on the day after removal of both adrenals proves nothing at all, especially in the absence of any check on the glycogen store. The isolated observation (in exper. 15) that the intravenous injection of a quantity of adrenalin (0.1 cc. adrenalin 1 in 200,000) which was inadequate to elicit the paradoxical pupil reaction yet caused a hyperglycemia, is cited as further evidence that in asphyxial hyperglycemia adrenalin plays an important part. There was already a hyperglycemia present on account of the subjection of the animal to anoxemia but the apparent slight increase in blood sugar in the specimen taken after injection of the adrenalin is definitely attributed to the 0.0005 mgm. of adrenalin injected.

Kellaway's acceptance of the statements in the literature that asphyxia increases the rate of output of epinephrin apparently accounts for the uncritical way in which he finds support in his own observations for the view that increased adrenalin output is a considerable factor in asphyxial hyperglycemia. He seems to forget that it would not be enough to prove that asphyxia causes an increase in the epinephrin output, it must be shown that the increase is sufficient to bring the epinephrin content of the blood to the level necessary for adrenalin hyperglycemia and to maintain it there.

In our own work (7), (8), (9), we have not found evidence that there is any detectable increase in the output per unit of time, although, of course, when the blood flow through the adrenals is slowed the concentration of epinephrin in it is increased. The reason why our result differs from that of Kellaway is that we used quantitative methods which really enabled us to measure the output of epinephrin whereas he did not make any measurements at all, but assumed from the effect of asphyxia in causing the paradoxical pupillary reaction that there must have been an increase in the output. This is not a specific qualitative reaction for adrenalin, let alone a reaction by which the rate of output can be quantitatively estimated.<sup>2</sup> The fundamental difference

<sup>2</sup> The pupil reaction can be utilized for estimating quantitatively the epinephrin in adrenal vein blood, and it has been so employed by us. But to do this variations in the other factors which may affect the pupil (anesthesia, asphyxia, etc.) must be eliminated, the only change made being the addition to the general blood of the epinephrin containing-adrenal blood collected in a cava pocket for a definite time or the adrenalin artificially injected to assay it.

between Doctor Kellaway's work and our own embraces much more than the single question of the effect of asphyxia on the epinephrin output. All our work on the influence of various conditions and various substances upon the epinephrin output has aimed at a quantitative determination of epinephrin in the adrenal vein blood. Being able to tell how much epinephrin the adrenals were giving off per minute before the factor under investigation was allowed to act, and how much they were giving off while it was acting, we were naturally in a different position for determining whether any change had occurred from that occupied by an observer who could not have any idea how much epinephrin was being given off at any time throughout his experiment.

He has shown that the anoxemia is the important factor in producing the paradoxical dilatation of the pupil in asphyxia. This is a new and interesting point. By graduating the severity of the asphyxia, as he terms it, i.e., by causing the animal to breathe mixtures with definite percentages of oxygen less than that of the atmosphere, he has satisfied himself that after interference with the epinephrin output it is less easy to provoke the paradoxical dilatation than in normal animals, although in non-anesthetized animals he still gets a fair reaction. He studied the difference produced in this reaction in cats by section of the splanchnics in survival experiments and by removal of both adrenals. We have no observations on cats after bilateral splanchnotomy. Our animals were prepared by excision of one adrenal and section of the nerves of the other according to Elliott's method, and we were unable to convince ourselves, as we have stated in a previous paper (10) "that there is any striking difference" in the paradoxical reaction induced by asphyxia in these animals as compared with normal cats, although "we should rather expect a difference if the normal epinephrin output," as we believe, "is already exerting an action" on the sensitized iris. Kellaway also obtained a relatively small difference between the normal and operated cats with the severer grades of asphyxia, and it is possible that by using his "graduated" method instead of the cruder methods previously employed a similar difference would be made out in cats prepared in the way mentioned as he found in cats after section of the splanchnics. But this, so far as we can see, would indicate merely that the epinephrin was a factor in the asphyxial paradoxical reaction and would not prove that the output was *augmented* by asphyxia.

We have obtained evidence (10) that epinephrin passing into the blood at the ordinary rate under the conditions of our experiments exerts an action on the pupil (after removal of the superior cervical ganglion). A dilatation produced by epinephrin disappears more quickly if the adrenal blood is prevented from entering the circulation. In a previous paper (9) we say that this being so "there is every reason to expect that asphyxia, which even according to Kellaway causes some paradoxical dilatation in the absence of the adrenals, will increase the reactivity of the pupil to this ordinary output." Kellaway professes to find that the phrase "every reason to expect" "does not appear to have any definite

significance." We should have thought it self-evident that since asphyxia, as such, that is to say in the absence of the adrenal epinephrin, can cause dilatation of the sensitised pupil and since epinephrin can also cause dilatation there would be every reason to expect that a given output of epinephrin would cause a greater effect when favored or reinforced by the action of asphyxia than in the absence of asphyxia, and that accordingly the fact that a paradoxical reaction was more easily elicited or elicited in greater strength with intact adrenals than in their absence would not of itself show that asphyxia augments the rate of output of epinephrin.

We have observed in non-anesthetised cats (some days after transection of the cord in the cervical region) that the giving of ether increased the reactivity of the pupil, sensitised by previous removal of the superior cervical ganglion, to adrenal vein blood collected in a cava pocket, so that with a given time of collection a reaction was obtained where none had been obtained before, or a good reaction was elicited where only a small one had been got before the ether. At first sight this looks like a proof that ether augments the epinephrin output even after cervical cord section. And a good many statements with no definite basis exist in the literature to the effect that anesthetics cause an accelerated output. All that was necessary to dispose of this interpretation of the experiment mentioned, was to inject a definite dose of adrenalin before and after administration of ether. The pupillary response to one and the same dose was increased by the ether. The explanation we think is obviously that ether, which itself causes a dilatation of the sensitised pupil, favors the dilating action of the adrenalin, just as asphyxia does. Depression of the pupillo-constrictor activity, however produced, would bring about such an effect, and Langley (11) points out that paralysis of the ciliary ganglion by nicotine, for example, may complicate observations made by the aid of the pupillary paradoxical reaction. Kellaway's experiments on the excised iris throw no light upon the question.

His observation that when the aorta is clipped asphyxia causes dilatation of both pupils, whereas on releasing the aorta there is a preferential dilatation of the sensitised pupil simply shows that enough epinephrin secreted at the ordinary rate has been collected in the adrenal vessels and the cava to give a good or a maximal pupillary paradox when it is allowed to move on after release of the aorta. The experiment is only a repetition in a crude form of our own observations on the measurement of the epinephrin output by the pupil reaction, and the paradoxical reaction is also obtained without asphyxia.

It is a complete mistake to impute our negative result in asphyxia to the condition of the animals in consequence of the operation practised by us to obtain the adrenal vein blood. *The paradoxical pupillary reaction is excellently obtained at the time when we are inducing asphyxia after having prepared the cava pocket for collection of the adrenal blood and thereafter in the course of the experiment.* According to Kellaway this proves that the epinephrin output is augmented. We collect the



adrenal blood at this time. We estimate the concentration of epinephrin in the blood and, knowing the rate at which the blood was collected, we calculate the output of epinephrin per minute and we find that the output has not been changed by the asphyxia. The "clear and logical conclusion" is not that the phenomenon of the acceleration of adrenalin secretion supposed to be evidenced by the pupillary reaction fails under the conditions of our experiments, but that no sensible acceleration is produced by such grades of asphyxia as we have employed.

This ought to be sufficient to dispose of the suggestion that the operative procedure adopted to obtain the adrenal blood vitiates our results. But a few words on the development of the technique may prevent misunderstanding. Permanent ligation of the abdominal aorta just above the bifurcation, of the renal arteries and veins and of the inferior cava was not practised until it was shown that it did not cause any demonstrable effect on the epinephrin output. In one of our earlier papers (7) it is stated under "technique" that "where the eye reactions are used alone the cava pocket need not be permanent. For certain purposes the temporary closing off of the pocket for a minute or two at a time is all that is necessary, and in the interval the circulation proceeds practically in the normal way. A clamp is applied just above the iliac veins. The renal veins are then clamped and the segment of cava emptied of blood by gently stripping it upwards. Finally a clamp is put on the cava above the adrenal veins. Only a few seconds are occupied in the adjustment of these clamps. Small veins entering the cava segment have been previously tied." It was then tested whether permanent ligation of these vessels made any difference in the results. No difference having been found we thereafter tied these vessels as a matter of routine, and in our papers where it is simply stated that "the cava pocket was formed" it is implied that the vessels tied were only the abdominal aorta, cava, renal arteries and veins and the small veins. For blood pressure assays it was sometimes found advantageous to tie the superior mesenteric artery and the coeliac axis to eliminate irregularities in the curve. Before this was done numerous observations were made which failed to reveal that the epinephrin output was at all affected by this procedure. We have continued to tie these vessels or sometimes only the superior mesenteric, in some of our experiments, because a higher blood pressure and better blood flow through the adrenals and their nervous mechanism are thus insured. Their ligation is not in any way inherent in our method and in all our investigations there are plenty of experiments in which they were not tied, for example, the last experiment on asphyxia published by us (9). In our large series of experiments on strychnine, nicotine and other drugs the superior mesenteric artery and coeliac axis were not ligated. We have also measured the epinephrin output in blood collected from one adrenal of a dog through a lumbar incision without opening the peritoneum and found no essential difference in the results from those of our other experiments.

## SUMMARY

1. Our previous conclusion that the adrenal epinephrin output is not essentially concerned in the hyperglycemia induced by ether narcosis and asphyxia is confirmed. In rabbits which have survived the removal of both adrenals and have recovered from the operation and whose livers are well filled with glycogen, hyperglycemia is caused by these procedures just as in normal rabbits. We reaffirm our position that for these forms of experimental hyperglycemia and for the hyperglycemia caused by piqûre the so-called adrenalin hypothesis should be abandoned.

2. We do not find that any real evidence has been adduced by Kellaway that the epinephrin from the adrenals has a demonstrable share in the production of asphyxial hyperglycemia. His experiments really confirm our conclusion that interference with the epinephrin output does not modify essentially the hyperglycemia caused by asphyxia.

3. Kellaway has produced no evidence that asphyxia (or anoxemia) causes a demonstrable increase in the rate of epinephrin output from the adrenals. Despite his statement that "the results of the different experiments fully justify the conclusion that the paradoxical pupil reaction is a good index of the epinephrin output," we do not believe that by looking at the eye of an intact cat he can tell the amount of epinephrin coming off from the adrenals per unit of time before, during or after asphyxia, whereas we can obtain these amounts by collecting adrenal vein blood and assaying its content of epinephrin. The real reason for the difference in our conclusions as to the influence of asphyxia upon the rate of epinephrin output is that we have attacked a quantitative problem by direct quantitative methods instead of trusting to ambiguous reactions which are not even specific qualitative reactions for epinephrin. Our data, therefore, have a very different value for the determination of changes in the rate of epinephrin output.

4. Kellaway is completely mistaken in imputing our results on the epinephrin output in asphyxia to the condition of the animals in consequence of the operation practised by us to collect the adrenal vein blood. The paradoxical pupillary reaction is excellently obtained at the time when we are inducing asphyxia after having prepared the cava pocket for collection of the adrenal blood and thereafter in the course of the experiment. According to Kellaway this proves that the epinephrin output is augmented at the very time when we are collecting the blood. We ought therefore to be able to detect the augmentation

by our method. Since we do not detect an increased output by assaying the very blood in which the epinephrin is carried we are compelled to conclude that Kellaway's interpretation of the pupillary reaction as demonstrating an increased output of epinephrin in asphyxia is erroneous.

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# THE EXPERIMENTAL PRODUCTION OF EDEMA AS RELATED TO PROTEIN DEFICIENCY

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## INTRODUCTION

The shortage of food in general and of certain foodstuffs in particular, which occurred as one of the results of the recent war, has been the cause of a number of forms of malnutrition among which is that called "war edema" or "war dropsy." This is a form of malnutrition that has been recognized only recently, and the characteristics of the type of diet that has been the cause of its development have been a subject of considerable speculation and some research.

Budzynski and Chechowski (1) report a condition of dropsy among the inhabitants of Poland in 1915. The principal articles of food were potatoes (which were often of bad quality), soup and bread, with little fat and practically no milk or meat. Landa (2) reports a great number of cases of edema during recent war conditions in Mexico, 1915. The diet was made up largely of spinach and beets. Jurgens (3) in comparing conditions in various concentration camps of prisoners of war has studied the relation of an "edema disease" to the ordinary infectious diseases of prison camps and finds that the edema is not a part of the infectious syndrome as had been considered by some. He states that it results from a dietary deficiency and that where this was recognized and a more varied diet was given, the men soon recovered from the edema or its appearance was averted. He further states that often the full syndrome did not appear until the men were set to digging. Then 10 to 20 per cent of the men developed the edema disease. Wells (4), during his investigations in Roumania, 1917, observed a number of cases of dropsy among the people who were most seriously deprived of food. He describes this condition to the lack of fats, particularly the

lack of those fats containing the "fat-soluble A." He states: "We found, as far as we had time to observe it, that when the patients were given butter fat they were likely to get better. But, of course, when under treatment they always got milk, soup and other things." Park (5), who spent some time in war prisons, made a study of the prisoners who received war rations. He ascribes the condition to underfeeding, especially of fats and proteins, and to the large amounts of fluid ingested together with the increased amount of NaCl. Jaksche (6) describes an edematous condition due to the hunger conditions in Austria. He states that the edema develops when the calorie intake falls below 1400 calories per day. Knack and Neumann (7), Maase and Zondek (8) and Maase and Zondek (9) describe an edema mostly in men over forty. This edema they say is intermittent according to the diet. They state that this nutritive disorder is due to the combination of overfeeding of carbohydrates together with underfeeding of fat and an abnormally large intake of water from carbohydrates and soups. They further state that 100 grams of fat per day and a stay in bed is followed by improvement. Kraus (10) describes this condition of malnutrition occurring especially in the large cities and institutions of Germany. He states that the decrease in the quantity of food is undoubtedly the principal and perhaps the only cause of the increase in the death rate. The diet is described as a monotonous, coarse diet, not rich or tasty, with a lack of condiments and vitamins which led to a loss of appetite and this in turn to inanition. The intake of food was from 800 to 1300 calories per day where 15 per cent was indigestible carbohydrate, with very little fat and at the highest 50 grams of protein. This diet was used by men who were doing hard work. Guillermin and Guyot (11) report the same conditions in Poland and other war zones. McGarrison (12), describing the pathogenesis of deficiency disease, reports that inanition leads to a state of adrenal hypertrophy. He suggests that such a change may be held to account in great measure, through blood pressure changes, for the occurrence of "war edema." In an editorial (13) of the Journal of the American Medical Association on "what renders a diet inadequate," the question of the importance of the consideration of salts in the production and study of edema is discussed. Rubner (14), describing the food conditions and their effect on the race, describes war "edema" and refers to the lack of adequate protein in the diet more than any previous author. Beyerman (15) describes edema in the Netherlands where the symptoms disappeared promptly upon addition of fresh vegetables to the diet. Jansen (16) describes

the edema and suggests that the cause is probably an increase in the permeability of the capillary endothelium which alters the exchange of fluids and salts between the tissues and the blood. Vandervelde and Contineau (17) describe an edema of the lower limbs of patients who were deported from Germany. He states that there was a lack of food, deplorable hygienic conditions, and that there were disturbances in the cardio-vascular apparatus, accompanied by anemia and that dyspnea was brought on with little exertion. Therapeutic measures were dietary improvements and rest which were followed by the disappearance of all symptoms. Romano Tonin (18) reports a hunger edema in which polyuria is a constant accompaniment. Reach (19) observed that many subjects with edema had atrophic testes or cryptorchism. He states that in twenty-six cases of war edema where dietary changes were of no benefit, testicular apotherapy was tried and in fourteen cases the patients were cured. Denton and Kohman (20), while working on the dietary qualities of carrots, found that dropsy occurred in a large percentage of rats fed on a carrot diet, when the proportion of nitrogen had been reduced by the addition of some non-nitrogenous foodstuffs, such as fat and starch. In a preliminary note (21) of the present piece of work the writer describes the cure of rats which had developed edema on the low-protein-carrot-diet, by the substitution of 18 per cent pure casein for 18 per cent corn starch in the diet, all other factors remaining the same.

#### METHODS

##### *Preparation of materials*

*Dried carrots.* The carrots that were used in the dry diets were washed, trimmed and ground in an ordinary meat grinder, spread on glass trays which were supported in a drying oven through which air was passed which was heated to 37° to 40°C. The carrots were then stored until they were used in the diet, but were not left to stand more than one or two months.

*Wet carrots.* Carrots used in the wet diets were prepared as for drying but were used fresh in the diets.

*Butter fat.* Good quality of fresh butter was heated from 90° to 95°C. in order to separate the proteins and water from the butter fat which was decanted off.

*Lard.* Leaf lard was obtained from the market and rendered.

*Casein.* Skim milk was purchased in six-gallon lots. This was diluted with an equal volume of distilled water. The casein was precipitated with hydrochloric acid (250 cc. to 10 liters of  $H_2O$ ) and filtered through cheesecloth. It was then ground with mortar and pestle and divided into six lots and each lot covered with 5 to 6 liters of distilled water slightly acidulated with acetic acid, and was left to extract for about 24 hours. The water was then filtered off through cheesecloth and the casein was ground in a mortar with pestle, diluted to 4 liters with distilled water and dissolved with sodium hydroxide (250 cc. of 50 per cent NaOH to 10 liters  $H_2O$ ). When dissolved the solution was diluted to 6 liters or more and the casein was again precipitated, this time with glacial acetic acid. The water was filtered off through cheesecloth. The casein was again dissolved, as above described, and filtered through filter paper with the aid of a suction filter. The solution was again diluted and the casein precipitated with hydrochloric acid. The casein was then filtered out and washed with distilled water to remove the acid. The water was removed with the suction filter. The casein was then ground in 95 per cent alcohol, covered with 3 liters of alcohol and left to extract 48 hours. This extraction was repeated three times with 95 per cent alcohol and twice with ether, filtering the alcohol and ether off each time with the aid of the suction filter and mixing well the casein each time with the fresh extraction solution. The casein was then dried in air and stored.

This careful method for the preparation of casein with repeated extractions with alcohol and ether was used in order to be sure that the casein contained neither of the vitamins, "fat-soluble A" or "water-soluble B." In some previous work commercial casein was used after extended washings with dilute acetic acid and extractions with alcohol and ether. Animals fed on a diet in which this casein was used showed a retarded growth but none of the other symptoms commonly attributed to the absence of the fat-soluble vitamins.

*Starch.* Kingsford's cornstarch was used throughout the experiments.

"Water-soluble B" was extracted from wheat germ by two extractions, using 25 grams of wheat germ and 250 cc. of 95 per cent alcohol for each extraction. The alcohol containing the "water-soluble B" was mixed and dried with the other ingredients of the diets that were fed dry, and was dried on the proper amount of cornstarch which was used in the diets that were fed wet.

		<i>Salts</i>	
Salt II		Salt III	
	<i>grams</i>		<i>grams</i>
Sodium chloride.....	0.50	Sodium chloride.....	0.1739
Ca lactate.....	1.57	Sodium sulphate.....	0.318
Ca $H_4(PO_4)_2$ .....	0.60	Sodium $H_2PO_4$ .....	0.347
		Potassium $H_2PO_4$ .....	0.954
Salt VI		Ca $H_4(PO_4)_2$ .....	0.540
Sodium chloride.....	0.50	Ca lactate.....	1.300
Ca lactate.....	1.5507	Fe lactate.....	0.118
Ca $H_4(PO_4)_2$ .....	0.6232		

Salt III is essentially the salt which was shown by McCollum (22) to supply all the necessary mineral for normal growth in rats when the diet was made up of purified food substances. Instead of  $MgSO_4$ , as used by him,  $Na_2SO_4$  was used to supply the same amount of sulphur. The change was made to reduce the amount of magnesium as carrots have a high magnesium content. Salt II is used to supply the minerals that are deficient in carrots. The two salt mixtures were used in such proportions as to make the salt content of the diet as nearly as possible the same as that of milk.

#### *Making up diets and feeding rats*

*Wet diets.* The cornstarch with the extract of wheat germ, butter fat and salt was weighed, distilled water was added and the mixture boiled to a clear stiff paste, while stirring constantly. The starch was boiled only a minute or two in order to break up the starch cells and make them more easily digested. To this paste was added the proper weight of fresh carrots, ground in an ordinary meat grinder. This mixture was made up every two days and kept in the ice box between feedings. Fifty grams of this wet food mixture were weighed out, recorded and given to each rat each day and the amount scattered and left in the dish was estimated on the next day and recorded. The amount left was not weighed because considerable moisture would evaporate and this made weighing quite as subject to error as estimation. The difference of the two weights was used as the food consumption of the rat for that day. As some of the animals scattered their food a great deal, considerable more evaporation took place in the case of the food of some animals than of that of others. Although this evaporation was considered in estimating back what the rat had not eaten, there was, no doubt, some unavoidable error in the estimation of the food consumption of the rats on this diet. Still it was a



method, with some degree of accuracy, to compare the relative amount of food consumed and a means by which one would detect any great variation in the food consumption of an animal, or any great difference among different animals. Knowing the calorie value of the ingredients which make up a diet, and the weight of the completed diet, the calorie value per gram of diet was determined by dividing the total calorie value by the total number of grams in the mixture. From this figure and the food consumption record of each rat the daily calorie intake of each rat was determined.

*Dry diets.* The cornstarch, butter fat and salts were weighed out, distilled water was added and the mixture boiled to a stiff paste. To this the alcoholic extract of wheat germ was added and thoroughly mixed. The proper weight of previously dried carrots was then added and the entire mass well mixed, making a very stiff paste. This was then divided into equal portions by means of two tubes, one about 4 inches long and about 1 inch in diameter, and the other just enough smaller to fit into the larger one closely. The larger tube was filled with the stiff paste. This was pushed out by means of the smaller tube which had one end closed by means of a close-fitting cork. The column of paste was then divided into four equal parts with a knife. These cakes of paste were put on a glass tray and dried in the oven described above and stored for use. Knowing the value in calories and the dry weight of the bulk of food made up, the value of each small cake, in calories and grams, was determined by dividing the value of the entire quantity by the number of cakes into which the mass was divided. A record was kept of the number of cakes eaten by each rat, and from this record the food consumption of each rat for each week of the duration of the experiment was determined.

#### *Care of the animals*

The animals used for the experiments were raised in this laboratory from stock rats kept for that purpose. These rats were fed a variety of foods—milk, mixed grain, fresh carrots, bread and occasionally meat. The animals were weighed and their cages changed and sterilized once each week.

The animals used for experimentation were taken at from about 50 to 60 grams and only when they had shown normal growth previous to being put on the experimental diet. Each experimental animal was kept in a separate cage and a record was kept of his food consumption.

The cages used were made entirely of metal. The trays were covered with paper. The cages were changed and sterilized by live steam once a week for the rats eating dry diets, and twice a week or more, as thought necessary, for those rats kept on a wet diet. The animals on the dry diet were supplied with distilled water. Those on the wet experimental diet were given no water as the water content of the food was quite high.

#### RESULTS

*General description of animals on a low protein diet.* When normal growing rats are put on the low-protein carrot diet, they stop growing at once and very soon show a gradual loss in body weight, more rapid for some animals than for others. They may lose as much as 30 to 33 per cent of their original weight in nine or ten weeks and 46 per cent in sixteen weeks. Along with this loss in weight there is a decrease in activity, not marked at first but in later periods of the experiment very decided. The animal sits quietly in the cage most of the time with its back humped up, its eyes only partly open, and head bent down between its fore legs. It is usually asleep except while eating. When moving about it seems dull, moves slowly, the more so as the experiment progresses.

The coat soon becomes rough. The hair becomes fuzzy and dirty looking as compared to that of the normal rat. If the animal lives for a long time on this diet the hair comes out in patches until sometimes almost the entire body is bare. The skin becomes dry and scaly and the animal is very subject to lesions on the tail, ears and nose. The lesions are scaly red patches on the exterior surface of these parts, are never wet and do not contain pus but are slightly elevated.

There is a great decrease in general resistance. While the stock rats and rats on an adequate diet have never been troubled with lice, it is very difficult to keep an animal on this diet free from lice. Any exposure to cold also usually leads to death while the normal animal does not suffer from the same exposure. The animal shows a great muscular weakness with subsequent wasting. Anemia is indicated by the pallor of the ears as contrasted with the pink ears of a normal animal. One factor of this anemia seems to be a decrease in blood volume, for it is more difficult to draw blood from such an animal than from the normal animal.

The duration of life of rats on the low-protein-carrot-diet varies among different rats from a very short time (2 weeks) to 3 months and

in some cases more. Toward the end of this time there is usually extreme weakness and loss of appetite. At this stage quite a number of the animals die without showing any edema. In a larger percentage of cases, however, an edema develops, this percentage in the rats on the wet diet being higher (86 per cent) than in the rats on the dry diet (55 per cent). (See table 3). This edema is usually detected first about the face, especially about the eyes and cheeks. This may be noticed in some cases several weeks before any extreme edema occurs. A more extreme type is an edema about the chest and fore legs. This can first be detected by a thickening of the skin. The animal may die in this stage, or the edema may disappear and return intermittently until death, or a very extreme edema may develop where as much as 10 cc., or occasionally more, of fluid, either gelatinous or liquid, may collect under the skin of the chest in the form of a large "blister." Even this extreme type of edema may often disappear and return intermittently on this diet, but a gain in body weight has never been noticed in this partial recovery; on the contrary, there is a decided weakening from one time to the next which finally leads to death unless the diet is corrected soon enough. In some animals the edema is in the form of a general anasarca, a fluid being collected in the serous cavities, both pleural and peritoneal. Except in extreme cases this could not be detected with certainty until after the death of the animal when the cavities were opened and the fluid pipetted out and measured. The amount of fluid varies from 0.5 to 2 or 3 or occasionally more cubic centimeters. Sometimes there is an abnormal amount of fluid in both cavities and sometimes in either one or the other.

Post-mortem examinations were made of all the animals that died as a result of being fed the low-protein-carrot-diet. In all cases there was a depletion of fat in the body. A very common finding was pneumonic lungs. There was a marked decrease in size of the testicles in the male, which was easily detected in the living animal. There was usually a congestion of the lymph glands especially those in the neck.

#### EXPLANATION AND DISCUSSION OF TABLES AND RESULTS

*The edema is not due to a deficiency of fats or fat-soluble vitamins. Wet diets.* The lack of fat and "fat-soluble-vitamin A" has been emphasized as much or more than any other factor as the cause of edema in the regions which were most seriously deprived of food as a result of

the recent war. This idea, no doubt, developed from the fact that there was a great shortage of fats and dairy products in these regions, and because it has been previously shown that xerophthalmia, a dryness of the conjunctiva, which eventually leads to blindness, and an edema of the eyelids, occurs in rats as a result of being fed a diet entirely free from or low in its content of the fat-soluble vitamins as reported by Mendel (23), McCollum (24), Steenbock (25), Funk (26) and others. Edema as it has been produced in rats in this laboratory, however, is much more extensive and entirely distinct from that which occurs as reported in xerophthalmia for it may occur subcutaneously, covering large areas or almost the entire surface of the body of the rat, and may take the form of a general anasarca, with large amounts of fluid (5 to 6 per cent of the body weight) collected in the serous cavities.

My experimental results apparently prove that this edema as produced in rats in this laboratory is not a result of the lack of the fats or the fat-soluble vitamins. The diet used in this work to produce edema in rats was made up largely of carrots, with additions of starch, fats and salts, carrots being the only source of protein. This diet was chosen because it was with such a diet that this edema was first produced in rats while studying the nutritive value of carrots (20).

Table 1 shows the results of feeding four groups of rats on diets in which the carrots and salt content was the same throughout and the caloric value was practically the same. The quantity of fat and starch was varied. The amount of each constituent of the diet is shown in grams and calories. In these diets fresh carrots were used, of which the caloric value is about 0.4 calorie per gram of fresh carrots. In diet I (X butter), 60 grams of butter fat were used, this supplying 1 gram of butter fat for every 63 calories of the diet. McCollum and Davis (22) have shown that 5 per cent butter fat in a diet of purified food-stuffs was sufficient for normal growth in young rats. This amount of butter fat used by them supplied 1 gram of butter fat for every 88 calories in the diet. The amount of fat supplied in X butter diet should be sufficient for rats.

In diet II (X lard) the fat was supplied in the form of lard, the same amount being used as was supplied as butter in the first diet. In diet III (no fat) no fat was supplied but the quantity of starch was increased. In diet IV (K + A - B) the butter was increased to twice the amount that was used in diet I (X butter). The caloric value was kept practically the same as in diets I and II by increasing the amount of starch where the fat was decreased. This increase of butter was made to

## GENERAL EXPLANATION OF TABLES

The symbol "K" wherever it occurs in the tables denotes 550 grams (dry weight) of carrots, 228 grams of starch and 120 grams of fat.

"x" butter or "x" lard denote diets in which there are 60 grams of butter or lard respectively.

"A" represents fat-soluble-A in 120 grams of butter and "B" denotes the alcoholic extract of 50 grams of wheat germ. The presence or absence of these vitamins in the respective diets is indicated by the presence of a + or - sign before the letter. For example, K + A - B means that the standard diet K (550 grams of dried carrots, 228 grams of starch, 120 grams of fat) contained fat-soluble-A but was deficient in water-soluble-B.

TABLE I

*Table of wet diets with results of feeding*

	I X BUTTER		II X LARD		III NO FAT		IV K + A - B	
	Grams	Calor- ies	Grams	Calor- ies	Grams	Calor- ies	Grams	Calor- ies
Carrots.....	4500	1800	4500	1800	4500	1800	4500	1800
Starch.....	360	1440	360	1440	456	1824	228	912
	BUTTER		LARD				BUTTER	
Fat.....	60	540	60	540	0	0	120	1080
Salt II.....	19.2	0	19.2	0	19.2	0	19.2	0
Salt III.....	16.4	0	16.4	0	16.4	0	16.4	0
Total.....		3780		3780		3624		3792
Number of rats on diet....	7		6		5		3	
Number of rats developing edema.....	3		3		2		3 only slight	
Per cent of rats developing edema.....	42½		50		40		100	

Table 1 shows the results of a study of the effect of certain fats and the fat-soluble vitamins on the production of edema in rats. Edema occurs in rats on all four of the diets so butter fat or the fat-soluble vitamins in butter does not prevent the edema, neither does it decrease the percentage of edema. Even when diet IV was fed with the butter fat content of 120 grams, all of these rats developed edema. The table also shows that fat is not the cause of the edema, for it occurred with and without fat in the diet.

insure sufficient intake of butter fat and fat-soluble-vitamine by the rats as their food consumption seemed to be somewhat less than that of the normal rat and it was thought that possibly not enough of the fat was obtained in the smaller food consumption.

The lower part of table 1 shows the number of rats fed each diet, together with the number and percentage of them that developed edema. It is of interest to note that where the greatest amount of butter fat was used the greatest percentage of rats developed edema and that, although there is little difference, the lowest percentage of cases of edema developed on the diet in which there was no fat supplied. This shows that fat and fat-soluble-vitamine do not prevent edema from developing in rats if the protein content of the diet is very low.

*The edema is not due to a deficiency of water-soluble vitamine or salts. Dry diets.* Table 2 shows the results of a series of experiments in which dried carrots were used in making up the diets. The water used in making up the diet was also evaporated so the diet was administered in a dry form. The carrots dried to practically 11.8 per cent of the wet weight, making the calorie value of the dried carrots 3.33 per gram of dried carrots. The same constituents were used as those used in the diets given in table 1 with the addition of an alcoholic extract of wheat germ in some of the diets. Although carrots have a considerable amount of the water-soluble vitamine this addition of alcoholic extract was made to insure a sufficient intake of this vitamine, for the lack of this vitamine is known to cause beri-beri which is often accompanied by more or less edema (28).

Diets V ( $K + A + B$ ) and VI ( $K + A - B$ ) each contain the large amount of butter fat used in diet IV of table 1. In addition diet V ( $K + A + B$ ) contains the alcoholic extract of 50 grams of wheat germ which supplied the extract of 1 gram of wheat germ for every 76 calories of the diet. McCollum (29) has shown that 3 per cent of wheat germ supplies sufficient water-soluble-vitamine for almost normal growth in young rats. This amount supplies one gram of wheat germ for every 133 calories in the diet. The amount of wheat germ used in the diets shown in table 2, together with the water-soluble-vitamine in carrots should supply enough of the water-soluble-vitamine to eliminate the lack of this vitamine as the cause of the edema which develops in these rats. This evidence is strengthened by comparing the percentages of edema on diets IX and X where 60 grams of butter fat are supplied in each but the water-soluble-vitamine is supplied only in X from which diet 33½ per cent of the rats developed edema whereas only 16½ per cent

TABLE 2  
Table of dry diets with results of feeding

	V K + A + B		VI K + A - B		VII K - A + B		VIII K - FAT + B		IX X BUTTER + B		XI X LARD		XII K + A + B + 2 X SALT	
	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories
Carrots (dry).....	550	1815	550	1815	550	1815	550	1815	550	1815	550	1815	550	1815
Starch.....	228	912	228	912	228	912	490	1960	360	1440	360	1440	228	912
	Butter		Butter		Lard				Butter		Lard		Butter	
Fat.....	120	1080	120	1080					60	540	60	540	120	1080
Salt II.....	19.2	0	19.2	0	19.2	0	19.2	0	19.2	0	19.2	0	38.4	0
Salt III.....	16.4	0	16.4	0	16.4	0	16.4	0	16.4	0	16.4	0	32.8	0
Extract of wheat germ.....	50	0	0	0	50	0	50	0	50	0	0	0	50	0
Total.....		3807		3807		3807		3775		3795		3795		3807
Number of rats on diet.....	6		6		1		2		6		3		2	12
Number of rats developing edema.....	3		2		0		0		1		1		0	2
Per cent of rats developing edema.....	50		33½		0		0		16½		33½		0	16½

Table 2 shows the results of a further study of edema in rats as produced by inadequate diets. The results given here are produced by feeding dry diets and supplying the rats with an unlimited amount of distilled water. In addition to a variation in the fats in these diets, the salts and the extract of wheat germ were varied. As in table 1, this table also shows that butter and the fat-soluble vitamin in butter do not alter the occurrence of edema in rats when there is a deficiency in protein. This table also shows that an increase in the salt content or the addition of the alcoholic extract of wheat germ does not influence or prevent the occurrence of edema.

developed edema on diet IX with no water-soluble-vitamine supplied. The percentage of rats developing edema as a result of the diets with lard or no source of fat, diets XII, VIII and XI, is again low but we cannot interpret this fact as meaning that fat is a cause of the edema, for in table 1 on diet III (no fat) 40 per cent of the rats developed edema. So the presence of "water-soluble B." does not prevent edema from developing in rats on a low protein diet. McClugage and Mendel (30) report very poor utilization of salts where a diet is high in its content of indigestible cellulose from vegetables. In order to insure a greater assimilation of salts from the diet of carrots, which is very high in its content of indigestible matter, a diet was prepared in which the quantity of salt was doubled, diet XII (K + A + B + 2x salt) the same as diet V (K + A + B) except for its salt content. The percentage of edema was not markedly altered by the higher salt content in the diet. As the retention of salts by the tissues is a very well-known cause of edema, it is of interest to note that the percentage of edema developing on this diet of double salt content is not increased.

*How does the water content of the diet influence the edema?* Another factor in the production of edema is the water intake. The total percentage of edema developing from the diets of table 1 (wet diets) which were about 84 per cent water, is 52.4 per cent. The total percentage of edema developing from the diets of table 2 (dry diet) is 23.7 per cent. As the two series of experiments were conducted at different times the rats could not be selected from the same litters. In order to study this factor more carefully, another series of experiments was conducted, the results of which are recorded in table 3. In addition to this factor the salts were again varied to control this factor more completely, and a control diet was used in which casein was added as a source of protein.

In table 3 diets V (a) and XII (a) (dry diets) are the same as diets V and XII, table 2, respectively. Diets XIII and XIV (wet diets) are the same as V (a) and XII (a) table 3 respectively except for the water content. Eighty-six per cent of the rats on the wet diets developed edema whereas only 55 per cent of the rats on the dry diets developed edema. This percentage is much higher than that of the earlier work shown in tables 1 and 2. This is explained by the fact that the course of the edema was not so well understood in the early work. This edema is intermittent and may come on in a short time and disappear in a day. If no special notice was made of that particular animal at the time of the edema the case was overlooked. In the later work the animals were



watched more closely, hence no cases of edema were overlooked which accounts for the higher percentage of edema in the later experiments. In addition to this difference there is a decided difference in the length of time before the edema develops on the dry and wet diets. Rats on the wet diets develop edema and die in from 9 to 13 weeks, most of

TABLE 3  
Table of dry and wet diets with results of feeding

	DRY				WET				DRY	
	V (a) K + A + B		XII (a) K + A + B + 2 X Salt		XIII K + A + B		XIV K + A + B + 2 X Salt		Control	
	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories
Carrots.....	550	1815	550	1815	4500	1800	4500	1800	550	1815
Starch.....	228	912	228	912	228	912	228	912	84	336
	Butter		Butter		Butter		Butter		Butter	
Fat.....	120	1080	120	1080	120	1080	120	1080	120	1080
Salt II.....	19.2	0	38.4	0	19.2	0	38.4	0	38.4	0
Salt III.....	16.4	0	32.8	0	16.4	0	32.8	0	32.8	0
Extract of wheat germ.....	50	0	50	0	50	0	50	0	50	0
Casein.....	0	0	0	0	0	0	0	0	144	576
Total.....		3807		3807		3792		3792		3807
Number of rats on diet.....	11		9		7		8		3	
Number of rats developing edema	5		6		7		6		0	
Per cent of rats developing edema {	45.4		66.7		100		75		0	
	55				86					

Table 3 shows several very interesting and conclusive results. First, when the diet contains a high water content the percentage of edema in rats which are fed the low-protein diet, is markedly increased, an increase from 55 per cent to 86 per cent. Second, a variation in the quantity of salts in the diet had no effect on the occurrence or percentage of edema. With the single salt portion 12 rats out of 18 developed edema. With the double salt portion 12 rats out of 17 developed edema. Third, and very important, the table shows that when a sufficient amount of an adequate protein replaces an amount of cornstarch, of equal caloric value, no edema developed in rats as a result of feeding this carrot diet. Furthermore, on examination of figure 2, it will be noted that rats fed this diet, (the same as the low-protein-carrot-diet except for the protein content), grow and reproduce normally.

them being dead at the end of the 11th week. Quite a large number of the rats on the dry diets live 18 or even 30 weeks. However, a small number die as early as the 9th week. So an increased water intake increases the number of cases of edema developing in rats on a low-protein-diet.

In case of food shortages, therefore, when it is necessary to use only limited amounts of protein or to use proteins of a poor quality, it is of practical importance to use these foods in a relatively dry form rather than in the form of soups as is usually done to make the food more "filling."

The salt content of the diets in table 3 did not change the percentage of cases of edema. Out of seventeen rats on the double salt diet, twelve developed edema. Out of eighteen rats on the lower salt diet, twelve rats developed edema.

*The edema is due to protein deficiency.* In the control diet 144 grams of casein replaced 144 grams of starch, the caloric value, vitamin content, fat and salts remained the same. It should be noted that there were no cases of edema developing on this diet. Furthermore, the growth curves were normal (see fig. 1) for the rats on this diet. The females produced normally but showed some difficulty in rearing their young. However, rat 304 (see growth curve, fig. 1) gave birth to eight young weighing 35 grams at birth. After twelve days they had not shown normal growth and well-being. At this time the litter was reduced to four. At the age of six weeks the average weight of the four young was 55 grams and at the age of eight weeks, when they themselves ate of the control diet, the average weight of the four rats was 92 grams. They were active and in general good condition. This shows that the low-protein-carrot-diet used throughout the work to produce edema was adequate except for its protein content, for when sufficient adequate protein was added, no edema occurred and the rats grew and reproduced normally.

This control diet with adequate protein supplied by the addition of casein to the low-protein-carrot-diet was also used as a cure for rats that had developed edema on the low-protein-carrot-diet. In a number of cases a cure of the edema resulted when the edematous rat was put on this diet with adequate protein content, and the rat in question would become more active, would show a rapid increase in weight and also soon increase his food consumption. As long as the rat remained on this diet the edema did not return, but as soon as it was put back on the low-protein-carrot-diet it would lose weight, general symptoms

of malnutrition would appear and after a time the animal would die, some of the rats developing edema the second time. The most interesting and striking case is that of rat 165 whose weight curve is shown in figure 1.

Rat 165 was put on diet V (K + A + B), table 2, and after eating this diet for 11½ weeks he had a large "blister" on the chest between the forelegs and showed a marked subcutaneous edema of the left

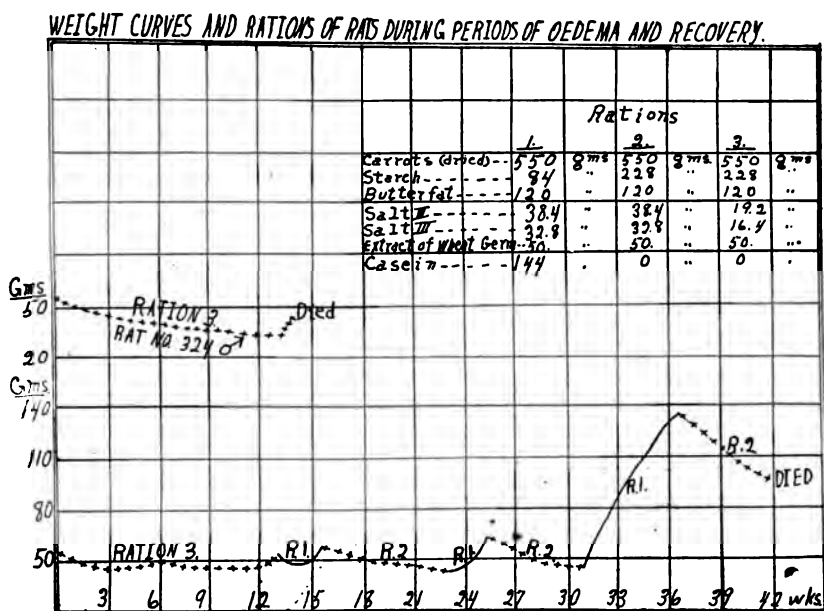


Fig. 1. The curve of rat 324 is a typical weight curve of rats kept on the low-protein-carrot-diet. There is a gradual loss in weight but when edema sets in a gain in weight occurs which is due to the accumulation of fluids in the tissues. No attempt was made to cure this rat.

The curve of rat 165 shows a gradual loss of weight while it was kept on the low-protein-carrot-diet, with an increase at the end of the 16th week due to the accumulation of fluid. Recovery followed a change in diet, the change consisting in giving adequate protein, with not much gain in weight, however. This animal developed a marked edema three times and was cured each time by a change to a diet containing adequate protein, a gain in weight and marked improvement in general condition resulting. During the last period of protein feeding, which was a relatively long period, the rat grew normally, its hair grew out perfectly over its entire body and it was very active. The rat finally died while on the low-protein-carrot-diet, without developing edema. At autopsy the lungs were found to be pneumonic, which was probably the cause of death.

side of the neck. After 3 days this edema had disappeared but after a few days it returned, the weight increasing from 45 grams to 52 grams due to the accumulation of fluid subcutaneously and in the tissues. At this time, 8 days after edema appeared for the first time, the rat was changed to the control diet (see table 3). In 2 days the edema about the chest and neck was entirely gone and only slight edema of the eyes remained, which cleared up in 2 more days. The weight of the rat had gone down to 47 grams. This finding is explained by the well-known observation of Bischoff and Voit (31) who noticed a decided loss in weight of a dog who had been kept on a diet of bread for 41 days, losing weight during this time; then when meat was administered in a quantity enough to cause a protein deposit, there was a decided loss in weight on the first day due to the loss of water.

After the above rat had been on this adequate diet a few days, increase in body weight followed but without any edema. This diet was continued for  $2\frac{1}{2}$  weeks when the rat was put back on the low-protein-carrot-diet but with the double salt portion. At the end of  $6\frac{1}{2}$  weeks the animal showed some slight signs of edema. This left but returned much more pronounced in a few days, again with a large "blister" on the chest. The rat was again returned to the control diet with adequate protein which was followed by disappearance of the edema in 14 hours. Again the animal became more active and showed rapid growth. His general appearance, however, was not normal for he had a great loss of hair and was not kept on the control diet with adequate protein long enough for new hair to grow. The animal was kept on this diet for 2 weeks during which time he gained 22 grams in body weight. He was again put back on the low-protein-carrot-diet with the double salt content. This was followed by the characteristic loss in weight and activity. During this period there was some growth of hair in the bare patches but the hair was not like the hair on the normal rat, it being very soft and fuzzy. After being on this diet for  $5\frac{1}{2}$  weeks the rat developed edema for the third time, this time about the neck and face but without the large "blister." The control diet was given, and normal growth was resumed as shown in the weight curve in figure 1. In 2 weeks there was rapid growth of hair which was firm and normal. There were some patches of the fuzzy dirty hair left which resulted from the poorer diet, but this hair was gradually replaced by normal hair. The rat was kept on this diet for 6 weeks and at the end of this time was in splendid condition, active, had fine coat of hair and eyes in excellent condition. The rat was put back on

the low-protein-carrot-diet and lost weight again, this time more rapidly. At the end of about 4 weeks the animal died without showing any signs of edema. He had been quite weak for some time and seemed to breathe with difficulty. After death the lymph glands were slightly congested, the lungs were congested and pneumonic. The cause of death was probably pneumonia which was contracted due to his low resistance as a result of the diet deficient in protein.

WEIGHT CURVES AND RATIOS OF CONTROL RATS.

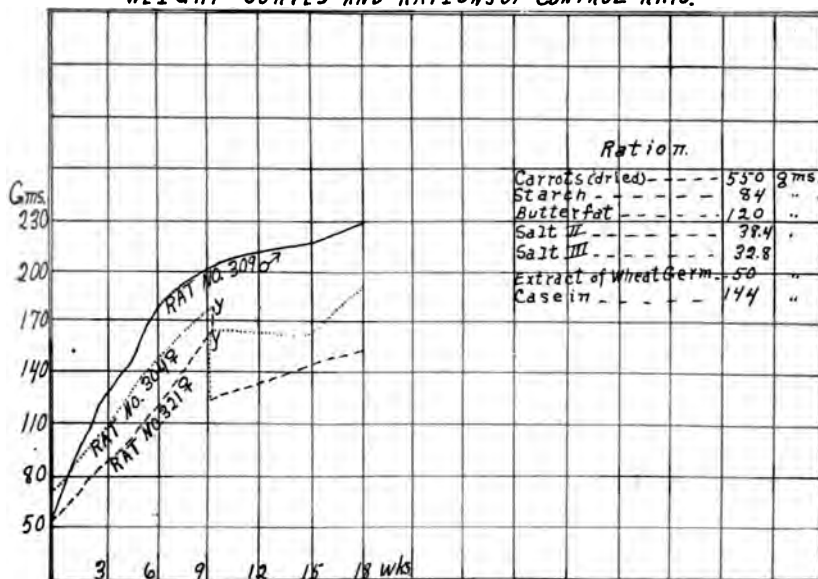


Fig. 2. The weight curves of the three control rats are given in this chart. These rats were fed the carrot diet containing an adequate amount of protein. (One is a male, the other two are females.) All showed normal growth and reproduction. The females gave birth to normal young (at *y*), but had some difficulty in rearing them. However, rat 304 succeeded in rearing a litter of young to the weaning stage and then the young ate the control diet and also grew normally with the control diet as their only source of food. These charts show that rats kept on a carrot diet with adequate protein do not develop edema.

Cure did not result in all cases of edema where the control diet was given to the rat. In a number of cases the rats were so feeble they refused to eat the food. The rats were usually left on the low-protein-carrot-diet until the edema had developed to an extreme degree and probably to such a degree that death would come before a sufficient

quantity of protein was assimilated. However, the writer feels confident that recovery would result in all cases if the diet were corrected soon enough, for in the control rats not only was edema averted, but none of the symptoms appeared that precede the edema where it develops.

In addition to the feeding done with the low-protein-carrot-diet, a lot of 3 rats was fed on a diet made up of 87 grams cornstarch, 5 grams butter fat, 4 grams of a complete salt mixture, 2 grams agar, 2 grams of casein and the alcoholic extract of 6 grams of wheat germ. This diet is adequate except for its protein content which makes up only 2 per cent of the diet. The general condition of the rats of this lot throughout the course of this experiment was about the same as that of the rats on the low-protein-carrot-diet, losing weight at about the same rate and having the same condition of hair and skin. One out of three of these rats developed edema just as those rats on the low-protein-carrot-diet did. The other two did not develop edema but showed the same signs of malnutrition.

*The edema is not due to the low-calorie intake of the edema rats.* Some observers have said that the edema that occurred in the war-stricken countries during the war was due to a low intake of calories. The food consumption records of my experimental animals show that the rats on the low-protein-carrot-diet did not consume as much of the diet as those who received adequate protein in their diets, although the same amount of food was given to each. Low calorie intake therefore had to be considered as a possible cause of the edema in these rats.

That low calorie intake is not the cause of the edema in the rats in this work is shown by figure 3, which gives the results of a study of this factor in the production of edema. Curve *I* is a composite weight curve of the three control rats which received 18 per cent of purified casein in their diet. Curve *III* shows the average weekly food consumption of these rats in calories. Curve *II* is a composite weight curve of six rats on the low-protein-carrot-diet. Curve *IV* shows the average weekly food consumption of these rats in calories. The only source of protein in the diet of the rats of curve *II* is the carrot in the diet. Curve *V* is a composite weight curve of five rats whose food consumption was limited in calories to that amount shown in curve *IV*. The diet of these rats was made up the same as that of the rats whose weight curve is represented by curve *II*, but with sufficient casein replacing starch in the diet to give each rat 1 gram of casein per day, which is approximately the amount of casein consumed by the control

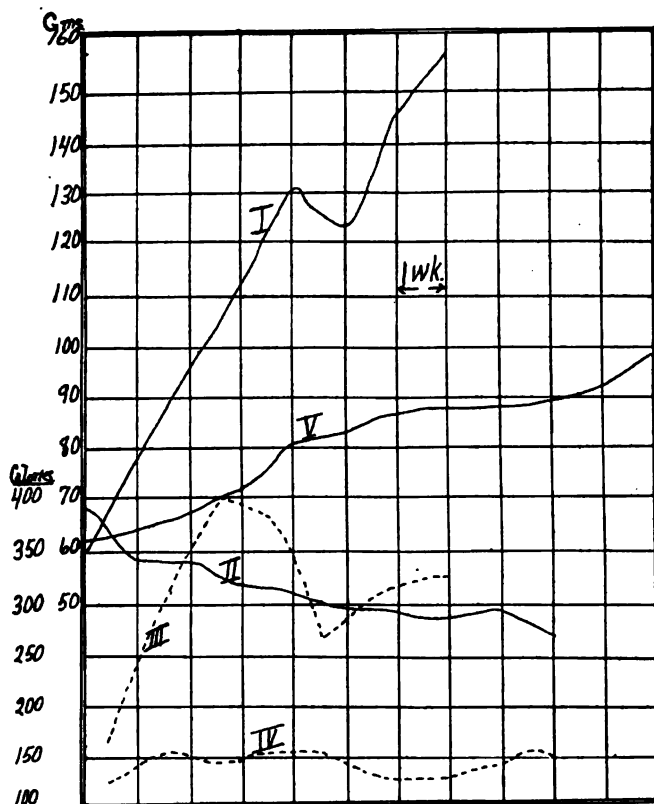


Fig. 3. The curves of figure 3 show that the low calorie intake of the rats used in this work is not responsible for the development of the edema.

Curve I is the composite growth curve of rats with 18 per cent casein in their diet. Curve III represents their average weekly food consumption in calories. Curve II is the composite weight curve of rats on the low-protein-carrot-diet. Curve IV represents their average weekly food consumption in calories. Curve V is the composite growth curve of rats whose food consumption is limited to that represented in curve IV, i.e., the same as the rats whose weight curve is shown by curve II, which means that the rats of curve V received a low-calorie diet; but the rats of curve V were fed adequate protein, 1 gram of casein per day being fed and cornstarch, of the caloric value of the gram of protein added, being withdrawn. So the rats in curve V received a low-calorie diet with adequate protein. These rats (curve V) did not show edema but slowly gained in weight. As pointed out before, the rats of curve IV showed a large percentage of edema. Which proves that the comparatively low-calorie intake of the edematous rats was not the cause of the edema, for edema did not develop in the rats (curve V) receiving adequate protein with low-calorie intake.

I. Composite weight curve of 3 rats on carrot + 18 per cent casein diet.

II. Composite weight curve of 6 rats on carrot—low-protein diet.

III. Curve showing average weekly food consumption in calories of rats of curve I.

IV. Curve showing average weekly food consumption in calories of rats of curve II.

V. Composite weight curve of 6 rats on low calorie diet with adequate protein.

rats. There is a slow gradual gain in weight of these rats which was still continuing at the time the experiment was stopped. The rats showed a slight tendency to the lesions described in the rats kept on the low-protein-carrot-diet, which shows that these lesions are not specific for the absence of adequate protein in the diet, but due to an infection as a result of lowered resistance. These lesions could be cured by the application of vaseline. With the exception of the lowered resistance and the retarded growth, these animals appeared to be in excellent condition, the hair and eyes being normal as well as appetite and activity. If the low calorie intake was the cause of the edema in rats, these rats should have at least shown some edema at the time the experiment was stopped, for they had been kept on this low protein diet 14 weeks. The diet was fed in the wet form. When rats of the same age and weight had been fed the wet low-protein-carrot-diet (results shown in table 3) the rats were all dead at the end of 13 weeks and a large percentage of them had developed edema.

In addition to the work recorded in figure 3, some rats were fed a diet of even lower calorie value, the food consumption being reduced by 16 per cent, but each rat was still receiving 1 gram of purified casein per day. Some of these rats lost weight and died without showing edema and some were growing and in splendid condition when the experiment was stopped. So the low-calorie intake of the edematous rats is not the cause of the edema.

*The edema is not due to some "toxic" effect of the carrots.* The writer has been told that the people of Germany who used vegetables as such a large part of their diet and suffered from war edema are said to have called the disease "Rübenkrankheit." The low-protein-carrot-diets which were the cause of the development of edema in these rats had a very high carrot content and some might say that there may be some toxic product in the carrots that impairs the kidney or some other tissue of the body and makes it impossible to eliminate fluids from the body. This cannot be the case, for the control rats receive the same proportion of carrots in their diets and are perfectly normal. Furthermore one lot of three rats was fed on a low-protein-diet (2 per cent casein) with no carrots, but with the calories, usually supplied by carrots, supplied by cornstarch. The rats followed the same general course and one developed edema just as the rats on the low-protein-carrot-diet.

*Why do not all of the rats develop edema and why is the edema intermittent?* It is difficult to explain why some of the rats on this low-protein-diet develop edema and others do not. There is no relation





Fig. 4. Rats 321 and 319 are from the same litter. The photograph was made after the rats had been feeding on experimental diets for eight weeks. Rat 321 received the control diet with adequate protein in the form of pure casein. It has grown normally (as shown by the photograph and by the growth curve in figure 2) and later reproduced normally. Rat 319 received the low-protein-carrot-diet and at the time the photograph was made was showing the early signs of edema, as shown in both the side view and front view of the rat. Its cheeks puff out and its eyes are partly closed. The normal rat was selected from the stock rats for a comparison of the normal shape of the face with the edematous face. It is a much younger rat than rat 319 but was selected because it was about the same size as No. 319. A comparison of the size of the eyes and the width of the face is quite striking.

between age or weight and the development of edema, neither is one litter more liable to edema than another. All of the rats on this diet grow weak and are quite similar in showing all the other symptoms of malnutrition. A muscular wasting is evident in all and an atrophy of the testicles of the male is quite evident. No doubt histological work would reveal atrophy in all the tissues. But why should the tissues of one rat undergo changes which cause them to retain fluids to this excessive degree and those of another rat not undergo these same changes, when both rats are from the same litter, have the same weight, and have the same environment and are receiving equal amounts of the same kind of food? Neither is there any relation between the amounts of food consumed and the development of edema; the food consumption varies among the rats that develop edema just as it varies among the rats that do not develop edema.

Another fact that is difficult to explain is the intermittency of the edema in many of the rats. This too is very irregular. Sometimes rats will show edema 6 or 8 weeks before their death. It disappears and may or may not return, or it may return and disappear several times. Very often rats will show an extreme edema, such as has been described as a "blister" on the chest and very often this disappears almost entirely in 12 to 24 hours, without any change in diet. Why do the tissues retain the fluid at one time and then change so suddenly? Could it be, perhaps, that the absence from the diet of sufficient amount of certain specific substances, whose absence is responsible for the development of the edema, causes the tissues generally or certain specific organs to undergo changes (as they do in actual starvation) which leads to the liberation of these specific substances and in turn to the temporary disappearance of the edema? No doubt a clearer knowledge of the tissue changes, that are responsible for the retention of the fluids, would throw some light on the subject.

*How does the acid content of the diet influence the edema?* An attempt was made to determine more accurately the changes that take place in the tissues to enable them to retain water to the striking degree that occurred in these edematous rats. As acidosis is a suggested cause of edema this was one of the first factors to be considered. The ordinary methods for determining this condition seemed difficult to apply in such small animals for it would be difficult to obtain sufficient blood without sacrificing the animal, which course did not seem practical in this stage of the work. As an indicative measure a diet was made using salt mixture VI instead of salt mixture II. Salt mixture VI has



Fig. 5. Rat 302 is a typical case of the edema in the form of a "blisters" on the chest. The hair of the rat was moistened with water and brushed down in order to give a better view of the large amount of fluid collected subcutaneously on the chest of the rat. This rat was very weak at the time the photograph was made and died about 12 hours later, but other rats with the same type of edema and of the same degree were cured by the administration of a diet of the same caloric but with an adequate protein content.

slightly more of the acid salt  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  in place of some of the calcium lactate as in salt mixture II. With the exception of this small change in the acid content this new diet was identical with diet XII (see table 3). As a control diet XII (a) was used. Of the rats fed this diet with increased acid content, 88 per cent (8 out of 9 rats) developed edema whereas on the control diet only 50 per cent (3 out of 6 rats) developed edema. It should be noted, however, that when diet XII (a) was fed earlier, as recorded in table 3, 66 $\frac{2}{3}$  per cent of the rats developed edema, a somewhat greater percentage than occurred in this experiment. These results would indicate that the increased acid content of the diet so alters or injures the tissues as to make them more likely to retain water. That the acid itself is not the cause of the edema is shown by the fact that one of the rats that had developed edema on this more acid diet was cured by putting him on a diet with the same acid content but with 18 per cent pure casein replacing 18 per cent cornstarch. This rat was not only cured but in the succeeding 5 weeks increased in body weight 26 grams. This strengthens the evidence for the curative value of adequate protein in cases of edema of this type since it is here successful in curing the edema with the adverse condition of the higher acid content present.

*The relation of the kidney to the protein deficiency.* There is some indication that the kidney cells are injured and therefore fail to eliminate the water. Although very little work was done on the kidney tissues, sections were made of a few kidneys and some albumin found in the tubules. Attempts were made to test the urine. It was difficult to collect uncontaminated urine from the rats so that the urine was collected, after death, from the urethra (where urine remained in the bladder) by slight pressure on the bladder. Positive tests for albumin were obtained but when the normal rats were killed and the urine collected in the same manner and tested, positive results were also found which made the above experiments of no value. And yet it seems probable that the albumin in the tubules would follow the current of urine into the bladder.

In this connection it is interesting to note the work of Epstein (32), who recommends a diet high in well-selected proteins and low in carbohydrates and fats, in cases of edema in certain types of chronic nephritis. He further suggests that chronic parenchymatous nephritis might be genetically a disorder of nutrition. Allbutt (33) reports a case of renal dropsy cured by a high protein diet.

It is reported by Captain McCay (34) that renal disease is more common among the population of Bengal than among the Europeans who consume more protein than the Bengali.

One of the long recognized results of a high protein diet is the impairment of kidney cells. Chittenden (35) emphasizes the importance of limiting the "daily intake of protein food to as low a level as is consistent with the true needs of the body, in those cases where the kidneys are at all enfeebled or where it seems desirable to exercise due precaution as a possible means of prevention." From the evidence here given it would seem that there is equally as great danger of renal impairment from a low protein diet. This follows very reasonably for **certainly there is a weakening of all the tissues**, quite evident in the general muscular **weakness** of the animal which is certainly due to a specific protein starvation of the cells.

It is reported (3) that often edema did not occur among people when on the restricted war diet, until they did hard work. It is further reported that rest in bed along with dietary changes was very beneficial. This would indicate that muscular work is a predisposing factor to the edema, which seems very probable. If the tissues of the body are not receiving enough food, certainly muscular work would relatively diminish the amount of food. Furthermore, during muscular work lactic acid is formed and according to Fischer (36) acid renders the tissues able to hold more water.

#### CONCLUSIONS

1. When young rats are fed diets composed largely of carrots and with carrots as the only source of protein, a large percentage of the rats develop edema.

2. Fats or fat-soluble-vitamine do not prevent the occurrence or decrease the percentage of edema in rats, even if 10 per cent of the calorie value of the diet is made up of butter-fat.

3. The water-soluble-vitamine does not prevent the occurrence or decrease the percentage of edema in rats fed the low-protein-diet.

4. Salts do not play any appreciable rôle in the production of this type of edema, for even when the salt content is doubled there is no noticeable effect upon the occurrence of edema.

5. The water intake of the animal which is feeding on the low-protein-carrot-diet decidedly influences the development of the edema. Edema develops more frequently, is more severe and develops sooner on a wet diet than on a dry diet.

6. When a sufficient amount of an adequate protein is added to the low-protein-carrot-diet, in place of an equivalent amount of cornstarch, edema is not only averted in rats feeding on this diet but the rats show normal growth and reproduction.

7. That the edema does not develop as a result of some toxic substance in the carrots is shown by the fact that rats fed on the control diet with the same carrot content as the low-protein-carrot-diet but with adequate protein did not develop edema and showed normal well-being.

8. When the acid content of the diet was increased the percentage of rats developing edema was increased from 50 per cent to 88 per cent. That the acid in this diet was not the chief etiological factor of the edema is shown by the fact that one rat on this diet was cured and made to grow normally on the diet with no change in the acid content but with an adequate protein supply replacing an equivalent amount of corn starch in the diet.

9. The edema manifested in these rats is not due to simple starvation, or low caloric intake, for when rats are fed a diet, including 1 gram of adequate protein each day, of the same caloric value as the diet fed the edematous rats, they do not develop edema.

10. This edema can be successfully cured by supplying the diet with adequate protein. Edema was produced and cured three times in one rat by alternating low-protein-diet (edema-producing) with an adequate protein diet.

11. These findings warrant the general conclusion that if it is necessary to limit the amount of protein in a diseased condition or in a period of national economic stress (as was necessary in some of the European countries during the recent war), it is advisable to administer the low protein diet in a form free from excess of water and any acid producing foods. Symptoms of developing edema must be looked for and adequate protein supplied immediately to effect a cure.

*Note 1.* I wish to express my deepest gratitude to Dr. A. B. Luckhardt for his keen interest and enthusiasm for the work throughout its course and for the many helpful suggestions that were offered from time to time; and to Edgar C. Turner for his great interest and excellent assistance rendered in taking care of the animals.

*Note 2.* The wheat germ used in all of these experiments was furnished by the Washburn-Crosby Milling Co.

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## CONTENTS

	PAGE
THE EFFECT OF THE SUBCUTANEOUS INJECTION OF ADRENALIN CHLORID ON THE HEAT PRODUCTION, BLOOD PRESSURE AND PULSE RATE IN MAN. <i>Irene Sandiford</i> .....	407
THE APPARENT INFLUENCE OF A DIET OF CARBOHYDRATES ON THE PANCREAS REMNANT OF PARTIALLY PANCREATECTOMIZED DOGS. <i>V. W. Jensen and A. J. Carlson</i> .....	423
THE COMPARATIVE PERFORMANCE OF MUSCLES SUBJECTED TO RHYTHMIC AND ARRHYTHMIC STIMULATION. <i>Harold A. Bulger and Percy G. Stiles</i> .....	430
RENAL ACTIVITY AND THE ACID BASE EQUILIBRIUM. <i>T. Nagayama</i> .....	434
THE UREA EXCRETING ACTIVITY OF THE KIDNEY AND PHOSPHATE EXCRETION. <i>T. Nagayama</i> .....	449
GASTRIN STUDIES. III. THE RESPONSE OF THE STOMACH MUCOSA OF VARIOUS ANIMALS TO GASTRIN BODIES. <i>R. W. Keeton, F. C. Koch and A. B. Luckhardt</i> .....	454
GASTRIN STUDIES. IV. THE RESPONSE OF THE STOMACH MUCOSA TO FOOD AND GASTRIN BODIES AS INFLUENCED BY ATROPINE. <i>R. W. Keeton, A. B. Luckhardt and F. C. Koch</i> .....	469
FURTHER OBSERVATIONS ON THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS, AND THE ACTION OF STRYCHNINE AFTER CERVICAL CORD SECTION. <i>G. N. Stewart and J. M. Rogoff</i> .....	484
THE RELATION OF CATALASE TO HEART ACTIVITY. <i>R. J. Seymour</i> .....	525
THE EFFECT OF VITAMINE DEFICIENCY ON VARIOUS SPECIES OF ANIMALS. I. THE PRODUCTION OF XEROPHTHALMIA IN THE RABBIT. <i>Victor E. Nelson and Alvin R. Lamb</i> .....	530
THE FLASHING INTERVAL OF FIREFLIES—ITS TEMPERATURE COEFFICIENT—AN EXPLANATION OF SYNCHRONOUS FLASHING. <i>Charles D. and Aleida v. t. H. Snyder</i> .....	536
EFFECT OF DIMINISHED OXYGEN UPON RATE OF NERVE CONDUCTION IN CASSIOPEA. <i>Alfred Goldsborough Mayor</i> .....	543
THE ALKALI RESERVE OF THE BLOOD PLASMA, SPINAL FLUID AND LYMPH. <i>J. B. Collip and P. L. Backus</i> .....	551
THE EFFECT OF PROLONGED HYPERPNOEA ON THE CARBON DIOXIDE COMBINING POWER OF THE PLASMA, THE CARBON DIOXIDE TENSION OF ALVEOLAR AIR AND THE EXCRETION OF ACID AND BASIC PHOSPHATE AND AMMONIA BY THE KIDNEY. <i>J. B. Collip and P. L. Backus</i> .....	568
IS THE LUMINESCENCE OF CYPRIDINA AN OXIDATION? <i>E. Newton Harvey</i> .....	580
A PHYSIOLOGICAL RESPONSE TO PITUITARY ADMINISTRATION. <i>F. S. Hammett, C. A. Patten and N. Suitsu</i> .....	588
INDEX.....	593

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## THE EFFECT OF THE SUBCUTANEOUS INJECTION OF ADRENALIN CHLORID ON THE HEAT PRODUCTION, BLOOD PRESSURE AND PULSE RATE IN MAN

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The results of previous studies of the effect on the respiratory exchange of the injection of adrenalin chlorid may be briefly summarized: An increase in the respiratory quotient was found by Fuchs and Roth (1), Hari (2), Bernstein and Falta (3), Lusk and Riche (4), (5) and by Tompkins, Sturgis and Wearn (6). La Franca (7), Wilenko (8) and Bernstein (9) found no change. A greater oxygen consumption or caloric output than normal was noted by La Franca (7), Fuchs and Roth (1), Bernstein and Falta (3), Bernstein (9), Lusk and Riche (4), (5), and by Tompkins, Sturgis and Wearn (6); Wilenko (8) found no change in the oxygen consumption and Hari (2) and Jackson (10) found a decrease.

Of these contributions the most important is that of Tompkins, Sturgis and Wearn (6). In a carefully controlled series of 34 experiments they obtained, without exception, an increase in the heat production following the injection of adrenalin chlorid (0.5 cc. of 1/1000) and this increase was usually accompanied by a rise in the respiratory quotient (27 experiments). Soldiers were studied; they were divided into three groups: *a*, Those with "irritable heart;" *b*, those with hyperthyroidism; and *c*, normal men, that is, well-trained active soldiers on full duty. There were 25 soldiers with "irritable heart," in 13 of whom the increase in the metabolism from the adrenalin injection was accompanied by an increase in the pulse rate and systolic blood pressure of at least ten points each, while in 12 there was a less marked increase. In the 3

TABLE 1

*The effect of the subcutaneous injection of adrenalin chlorid on the blood pressure, pulse rate, respiration rate, ventilation rate, respiratory quotient, total calories per hour and basal metabolic rate (standard dose of adrenalin chlorid 0.5 cc.)*

GROUP	CASE	DIAGNOSIS	SEX	AGE	WEIGHT kgm.	HEIGHT cm.	BLOOD PRESSURE				PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC FIRE- TION RATE		RES- VENTILATION RATE		NOTES			
							Basal		Highest		Percentage increase over basal in systolic	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal		Highest		
							Systolic	Diastolic	Systolic	Diastolic																
1	263084*	Exophthalmic goiter	M	28	57.6	176.4	154	70	7	121	128	6	137.6	143.3	4	0.75	0.80	+103	+111	23	25	10.06	11.14	Dosage of adre- nalin less than standard		
	247994*	Exophthalmic goiter	F	36	41.7	157.5	126	85	23	131	156	19	92.6	113.1	22	0.83	0.95	+85	+126	20	24	6.56	9.31	Dosage of adre- nalin less than standard		
	259130	Exophthalmic goiter	M	40	67.5	179.1	163	85	178	75	9	118	139	18	125.4	150.7	20	0.77	0.88	+76	+112	22	26	10.60	12.89	
	258885	Exophthalmic goiter	M	34	58.5	178.4	156	90	28	94	128	36	118.9	161.0	35	0.71	0.91	+74	+136	24	26	9.03	15.22			
	260316	Exophthalmic goiter	M	32	52.3	167.6	136	80	22	90	118	31	107.0	128.2	20	0.76	0.83	+71	+105	22	26	7.82	10.10			
	262154*	Exophthalmic goiter	F	37	41.4	164.2	136	70	3	106	135	27	85.3	101.1	19	0.75	0.90	+66	+96	17	26	6.48	8.88	Dosage of adre- nalin less than standard		
	262154*	Exophthalmic goiter			44.0		120	75	135	78	13	110	129	17	69.4	77.5	12	0.73	0.79	+31	+47	8	10	4.70	5.96	Dosage of adre- nalin less than standard; 14 days after thy- roidectomy
	258794	Exophthalmic goiter	F	51	43.4	156.9	130	70	155	70	19	123	141	15	77.4	95.3	23	0.75	0.85	+90	+97	16	28	5.71	7.24	
260358	Exophthalmic goiter	F	26	47.5	162.6	130	64	146	80	12	112	138	23	86.6	97.5	13	0.78	0.88	+58	+78	20	24	6.14	7.94		

261849	Exophthalmic goiter	F 24	49.1	163.7	120	72	146	78	22	92	114	24	66.8	79.7	19	0.79	0.86	+20	+43	14	4.98	6.27	
262089	Exophthalmic goiter	M 20	61.1	161.9	128	78	138	85	8	104	112	8	77.4	85.1	10	0.67	0.76	+18	+30	20	5.45	7.07	
262089*	Exophthalmic goiter		59.3		120	78	141	77	18	104	112	8	75.6	90.8	20	0.72	0.82	+17	+40	22	5.84	7.39	Thyroxin 5 days previous to this test
261192	Exophthalmic goiter	F 19	55.5	163.2	120	80	146	68	22	97	107	10	68.2	79.5	17	0.81	0.82	+13	+32	22	6.00	7.66	
261192*	Exophthalmic goiter		55.4		128	80	154	86	20	105	135	29	73.8	88.2	20	0.84	0.96	+22	+46	28	7.50	9.38	Thyroxin 5 days previous to this test
260662	Exophthalmic goiter	M 18	59.9	168.3	116	82	119	76	3	80	80	0	74.9	85.1	14	0.84	0.84	+9	+24	19	5.18	6.02	
245549	Adenomas of thyroid; hyperthyroidism	M 44	76.1	163.2	135	80	148	82	10	84	144	71	83.9	103.4	23	0.84	0.96	+20	+48	17	6.40	8.32	
245549*	Adenomas of thyroid; hyperthyroidism		74.4		138	65	154	66	13	96	116	21	81.1	97.9	21	0.80	0.93	+16	+41	18	5.79	7.98	Thyroxin 7 days previous to this test
261171	Adenomas of thyroid; hyperthyroidism	F 59	60.5	161.3	152	92	158	76	4	63	79	25	65.3	79.2	21	0.89	0.92	+14	+38	18	6.67	7.94	
262353	Adenomas of thyroid; hyperthyroidism	M 48	73.0	176.5	125	75	145	75	16	86	116	35	82.0	103.9	27	0.77	0.98	+12	+42	15	5.67	9.67	

TABLE 1—Continued

GROUP	CASE	DIAGNOSIS	SEX	AGE	WEIGHT kgm.	HEIGHT cm.	BLOOD PRESSURE				PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRA- TION RATE		NOTES		
							Basal	Systolic	Diastolic	Highest	Percentage increase over basal in systolic	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal		Highest	Basal
4	119415	Double oöphorecto- mized neu- rasthenic; hyperthy- roidism	F	21	45.5	164.4	114	74	130	70	14	74	112	51	62.7	83.2	33	1.06	1.17	+15	+53	16	22	6.72	12.10
5	287796	Adenomas of thyroid without hy- perthyroid- ism	F	30	59.5	174.9	125	75	118	74	-6	82	104	27	65.4	72.3	11	0.71	0.77	+4	+15	18	18	5.57	6.64
6	256032	Colloid goiter; hypothy- roidism	F	17	47.2	153.7	116	70	132	80	14	62	78	26	48.0	61.6	28	0.90	0.88	-16	+9	15	20	3.69	5.20
7	262125	Addison's dis- ease?	F	32	53.7	170.2	98	70	104	80	6	100	95	-5	57.3	66.3	16	0.82	0.80	-3	+12	14	16	5.34	5.63
	261437	Addison's dis- ease?	F	35	32.2	158.1	80	58	86	52	8	94	99	5	41.3	47.9	16	0.83	0.80	-8	+7	17	17	4.20	5.01



235194	Hypopituitar- ism	M 33	79.5	193.4	122	86	132	90	8	64	91	42	77.7	93.2	20	0.77	0.97	-6	+13	8	13	5.05	6.99	
261342	Hypopituitar- ism	M 33	67.4	177.8	90	64	96	62	7	62	68	10	63.4	72.0	14	0.72	0.77	-13	-1	16	18	4.58	6.17	
261342*	Hypopituitar- ism		64.7		116	78	118	66	2	58	90	55	68.2	82.9	22	0.65	0.85	-5	+16	16	18	4.20	7.00	Thyroxin 6 days previous to this test
276369	Hypopituitar- ism	M 44	62.8	173.2	90	60	88	64	-2	57	64	12	48.0	52.2	9	0.82	1.04	-28	-22	12	18	4.61	6.61	
262555	Myxedema	M 39	64.0	179.7	114	88	122	70	7	60	59	-2	42.9	47.2	10	0.83	0.85	-40	-34	10	13	3.25	3.06	
262555*	Myxedema		59.2		128	80	160	90	25	64	79	23	64.4	79.2	23	0.79	0.88	-7	+15	16	18	5.06	6.23	Thyroxin 7 days previous to this test
262235	Myxedema	F 49	68.6	160.6	120	90	132	85	10	73	76	4	43.5	48.0	10	0.82	0.81	-30	-23	10	9	2.74	3.06	
256013*	Myxedema	F 46	84.7	155.6	104	68	124	66	19	87	98	13	65.8	78.5	19	0.74	0.82	-1	+19	22	30	5.90	8.05	Thyroxin 19 days previous to this test
61699	Postoperative myxedema	F 41	54.1	163.2	130	86	112	80	-14	70	76	9	49.5	60.5	22	0.93	1.06	-13	+7	17	9	3.91	5.19	
137062	Postoperative myxedema	F 37	74.5	162.6	118	90	135	90	14	77	88	14	54.2	73.7	36	0.79	0.85	-18	+12	17	19	4.33	6.52	
257217	Atypical hy- pothyroid- ism on thy- roxin	M 56	52.3	173.4	122	68	132	68	8	65	96	48	66.7	94.5	42	0.78	0.89	+10	+55	14	17	4.73	6.58	
276977	Anorexia ner- vosa; hypo- thyroidism	F 16	37.8	170.6	80	58	104	52	30	79	84	6	43.5	46.8	8	0.84	0.85	-22	-16	18	20	4.02	4.61	

TABLE 1—Continued

GROUP	CASE	DIAGNOSIS	SEX	AGE	WEIGHT kgm.	HEIGHT cm.	BLOOD PRESSURE				PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRA- TION RATE		NOTES	Thyroxin 5 days to previous this test	
							Basal		Highest		Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal			Highest
							Systolic	Diastolic	Systolic	Diastolic															
3	261292	Neurasthenia	M	23	77.1	177.1	120	80	140	80	17	83	95	14	79.8	100.3	26	0.77	0.80	+4	+30	9 11	4.81	6.37	
	261292*	Neurasthenia			76.8		138	76	133	80	-4	99	108	9	97.7	116.4	19	0.80	0.87	+28	+52	12 10	5.74	7.70	
	280559	Neurasthenia	F	26	46.3	151.3	106	76	120	68	13	88	104	18	56.3	62.3	11	0.71	0.77	+9	+21	18 20	4.42	5.15	
	287871	Neurasthenia	F	23	72.6	160.6	110	85	118	80	7	89	96	8	65.3	72.2	11	0.83	0.86	+1	+11	15 19	5.04	6.01	
3	281027	Chronic nerv- ous exhaus- tion	F	21	52.0	169.5	104	68	112	64	8	78	96	23	55.8	64.7	16	0.75	0.84	-5	+10	19 24	4.63	6.19	
	281509	Chronic nerv- ous exhaus- tion	F	32	42.2	153.1	108	68	140	74	3	83	97	17	58.6	86.9	48	0.83	0.98	+20	+78	21 39	5.16	12.76	
4	165324	Normal control	M	22	70.5	174.7	118	78	142	78	20	77	104	35	79.7	99.3	25	0.73	0.89	+9	+38	12 18	4.95	7.44	
	283723	Normal control	M	47	68.8	175.0	128	80	140	70	9	67	84	25	79.5	88.7	12	0.82	0.81	+12	+25	18 17	6.20	6.74	
	283068	Normal control	M	37	67.8	172.9	130	82	140	70	8	84	88	5	70.0	85.3	22	0.90	0.83	-2	+19	19 16	7.11	8.69	
	283661	Normal control	M	25	70.5	178.3	104	65	124	74	19	60	73	22	79.3	94.6	19	0.77	0.82	+7	+27	16 21	5.44	7.69	

\* The cases starred are omitted from the group averages given in Table 2.

cases of hyperthyroidism there was an increase of more than ten points in both the pulse and systolic blood pressure, while in the 6 normal soldiers on active duty the response in the pulse rate and systolic blood pressure was less definite.

Before the publication of the paper by Tompkins, Sturgis and Wearn, an investigation on the effect of the subcutaneous injection of adrenalin on the heat production, pulse rate, and blood pressure had been started in this laboratory; a preliminary report has recently been made (11) of the results given here in detail. The method of procedure was as follows: The patients came to the laboratory in the so-called post-absorptive condition and the basal metabolic rate, pulse rate, and blood pressure were determined after the patients had rested in bed twenty minutes according to the regular routine practiced in this laboratory and described in detail elsewhere (12). For the determination of the basal metabolic rate a mask is tightly adjusted over the patient's mouth and nose and, by means of expiratory and inspiratory valves, the total volume of the patient's expired air is collected in a gasometer for a known period of approximately ten minutes. Duplicate determinations are made of the carbon dioxide and oxygen content of the expired air, the analyses being done in the Haldane gas analysis apparatus. From the data obtained the basal metabolic rate is calculated by the usual method.

After the various basal values have been determined, 0.5 cc. of 1/1000 solution of adrenalin chlorid (Parke, Davis & Company) is injected subcutaneously in the patient's arm; the pulse and blood pressure are taken every five minutes for the next two hours and the metabolic rate determined for approximately ten-minute periods beginning about ten minutes, thirty minutes, one hour, and one and one-half hours after the injection.

Forty-six experiments were done on the 39 patients grouped according to diagnosis in table 1. The results of these experiments are summarized in table 2. Four experiments in group 1 are not included in the average given in table 2 because the dose of adrenalin was less than 0.5 cc.; 2 experiments in group 2, 1 in group 3, 1 in group 8, 2 in group 9 and 1 in group 13 are not included in the average because of previous thyroxin administration. The experiments omitted from the average are starred in table 1. The data obtained as basal before the adrenalin injection are contrasted in table 1 with the data which showed the most pronounced reaction following the injection. The percentage increase over the basal is given for the total calories, pulse rate, and systolic blood pressure. In table 2 are given the average data for the

various groups of cases studied. In table 3 are grouped those cases in which the adrenalin test was done both before and after the patients were given thyroxin.

TABLE 2

*Summary of the effect of the subcutaneous injection of adrenalin chlorid on the systolic blood pressure, pulse rate, respiration rate, ventilation rate, respiratory quotient, total calories per hour and basal metabolic rate (standard dose of adrenalin chlorid 0.5 cc.)*

GROUP	NUMBER OF CASES	DIAGNOSIS	AVERAGE B. M. R. FOR EACH GROUP	PERCENTAGE VARIATIONS FROM BASAL			NUMERICAL VARIATIONS FROM BASAL			
				Systolic blood pressure	Pulse rate	Total calories per hour	Respiration rate	Ventilation rate: liters B. T. P. D.	Respiratory quotient	Metabolic rate
			per cent	per cent	per cent	per cent				
1	9	Exophthalmic goiters; five with B. M. R. above +50 per cent.....	+68	18	25	22	52.82	0.12	38	
2		Four with B. M. R. below +50 per cent.....	+15	14	11	15	21.35	0.04	17	
		Average of all exophthalmic goiters...	+44	16	18	19	42.17	0.08	29	
3	3	Adenomas of thyroid; hyperthyroidism	+15	10	44	24	32.40	0.12	27	
4	1	Double oophorectomised neurasthenic with hyperthyroidism .....	+15	14	51	33	65.38	0.11	38	
5	1	Adenoma of thyroid without hyperthyroidism.....	+4	-6	27	11	01.07	0.06	11	
6	1	Colloid goiter; hypothyroidism .....	-16	14	26	28	51.51	-0.02	25	
7	2	Addison's disease.....	-6	7	0	16	10.55	-0.03	15	
8	3	Hypopituitarism.....	-16	4	21	14	41.84	0.16	12	
9	2	Myxedema .....	-35	9	1	10	10.37	0.01	7	
10	2	Postoperative myxedema.....	-16	0	12	29	-31.74	0.10	25	
11	1	Atypical hypothyroidism on thyroxin.	+10	8	48	42	31.85	0.11	45	
12	1	Anorexia nervosa; hypothyroidism ...	-22	30	6	8	20.59	0.01	6	
13	5	Neurasthenia and chronic nervous exhaustion.....	+6	10	16	22	62.48	0.07	24	
14	4	Normal clinical controls.....	+7	14	22	20	21.72	0.03	20	

## DISCUSSION OF RESULTS

*Heat production.* In every experiment there was an increase in the heat production after the subcutaneous injection of adrenalin chlorid, the maximum occurring as a rule within ten to thirty minutes after the

injection. With but three exceptions, one patient with severe exophthalmic goiter in whom only 0.1 cc. adrenalin was injected, one patient with hypopituitarism, and one patient with anorexia nervosa, this maximum increase was at least 10 per cent above the basal; the greatest reaction was 48 per cent above the basal and occurred in a case of chronic nervous exhaustion. Within one and one-half to two hours after the adrenalin was injected the metabolic rate had returned to within 4 points of the preliminary level in 21 experiments; it was still somewhat elevated in 19 experiments, and it was slightly lower than the basal

Typical curve of the percentage increase in the total calories following the injection of adrenalin chlorid. Diagnosis: Neurasthenia.

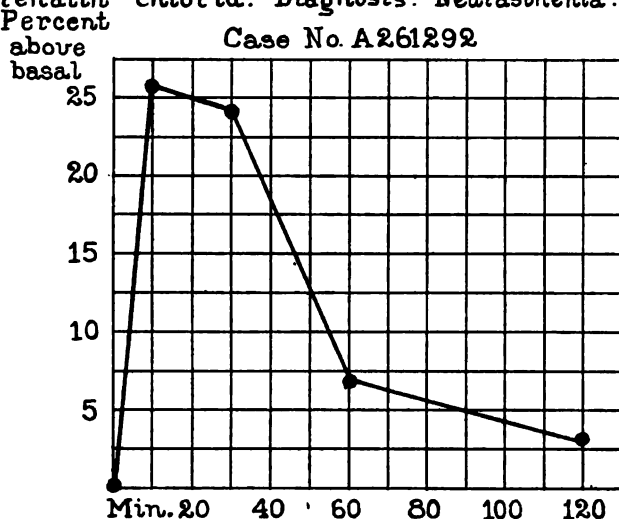


FIG. 1

determined before the injection in the remaining 6 experiments. A typical example of the post-adrenalin metabolic curve is given in figure 1.

In the group of patients with exophthalmic goiter with basal metabolic rates above +50 per cent who were given the standard dose of adrenalin the total number of calories per hour increased on the average 22 per cent over the basal, while in the group of patients with a milder degree of exophthalmic goiter whose basal metabolic rates were less than +50 per cent the total number of calories increased on the average 15 per cent. In spite of this no consistent relationship can be seen in the

TABLE 3

The effect of the subcutaneous injection of adrenalin chlorid, both before and after the intravenous injection of thyroxin, on the blood pressure, pulse rate, respiration rate, ventilation rate, respiratory quotient, total calories per hour and basal metabolic rate (standard dose of adrenalin chlorid 0.5 cc.)

CASE	DIAGNOSIS	BLOOD PRESSURE				PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRATION RATE		VENTILATION RATE LITERS—S. T. P. D.		NOTES							
		Basal		Highest	Percentage increase over Basal in systolic	Basal	Highest	Percentage increase over Basal	Basal	Highest	Percentage increase over Basal	Basal	Highest	Basal	Highest	Basal	Highest	Basal	Highest								
		Systolic	Diastolic	Systolic			Diastolic			Systolic			Diastolic		Systolic		Diastolic		Systolic		Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
262089	Exophthalmic goiter...	128	78	138	85	8	104	112	877.4	85.1	100.67	0.76	+18	+30	20	22	5.45	7.07			8 mgm. thyroxin 5 days previous to test						
		120	78	141	77	18	104	112	875.6	90.8	200.72	0.82	+17	+40	22	26	5.84	7.39									
261192	Exophthalmic goiter..	120	80	146	68	22	97	107	1068.2	79.5	170.81	0.82	+13	+32	22	32	6.00	7.66			8 mgm. thyroxin 5 days previous to test						
		128	80	154	86	20	105	135	2973.8	88.2	200.84	0.96	+22	+46	28	34	7.50	9.38									
245549	Adenomas of thyroid; hyperthyroidism....	135	80	148	82	10	84	144	7183.9	103.4	230.84	0.96	+20	+48	17	16	6.40	8.32			8 mgm. thyroxin 7 days previous to test						
		136	65	154	66	13	96	116	2181.1	97.9	210.80	0.93	+16	+41	18	22	5.79	7.98									
261342	Hypopituitarism.....	90	64	96	62	7	62	68	1063.4	72.0	140.72	0.77	-13	-1	16	18	4.58	6.17			5 mgm. thyroxin 6 days previous to test						
		116	78	118	66	2	58	90	5568.2	82.9	220.65	0.85	-5	+16	16	18	4.20	7.00									
262555	Myxedema.....	114	88	122	70	7	60	59	-242.9	47.2	100.83	0.85	-40	-34	10	13	3.25	3.66			5 mgm. thyroxin 7 days previous to test						
		128	80	160	90	25	64	79	2364.4	79.2	230.79	0.88	-7	+15	16	18	5.06	6.23									
261292	Neurasthenia.....	120	80	140	80	17	83	95	1479.8	100.3	260.77	0.80	+4	+30	9	11	4.81	6.37			5 mgm. thyroxin 5 days previous to test						
		138	76	133	80	-4	99	108	997.7	116.4	190.80	0.87	+28	+52	12	10	5.74	7.70									

various groups studied and summarized in table 2, between the height of the adrenalin reaction and the degree of hyperthyroidism or hypothyroidism as determined by the level of the metabolic rate. This inconsistency is further shown in the 6 cases, (table 3) in which the adrenalin reaction was studied both before and after the intravenous injection of thyroxin for in two of these the percentage increase in the total calories after the adrenalin injection was less instead of greater in the adrenalin test carried out after the patients had received thyroxin. Furthermore, there is only a very slight difference in the average increase in the heat production from the adrenalin injection before thyroxin was administered (17 per cent) as compared with the average increase after thyroxin was given (21 per cent).

The metabolic curve resulting from the subcutaneous injection of adrenalin is entirely different from that found by Plummer following the intravenous injection of 16 mg. of thyroxin. Following the injection of adrenalin the height of the metabolic curve is reached in approximately from ten to thirty minutes and has returned to its basal level in about two hours; when thyroxin is injected, however, the height is not reached for approximately from three to ten days and the basal level not regained for from one to two months. The presence of hyperglycemia (13) following the injection of adrenalin chlorid naturally directs attention to the similarity of the post-adrenalin metabolic curve to that found by Lusk (14) as a result of carbohydrate plethora following the ingestion of glucose.

*Ventilation and respiration rates.* There is invariably an increase in the ventilation rate following the injection of adrenalin chlorid and this is usually accompanied by an increase in the respiration rate, although there may even be a decrease in the latter. The variations in the percentages of carbon dioxid eliminated in the expired air and of oxygen absorbed from the inspired air vary inversely with the changes in the ventilation rate and directly with the increase in the metabolism. It is impossible, however, to predict the degree with which the increase in the respiratory volume will predominate over the concentration of that volume in different individuals or even in the same individual at different times.

*Respiratory quotient.* Following the injection of adrenalin there was an increase in the respiratory quotient in 39 experiments, no change in 1 experiment, and a decrease in 6. These results taken in conjunction with those of Tompkins, Sturgis and Wearn (6) indicate that as a rule there is an increase in carbohydrate combustion; this is confirmed by

the findings of other observers of an increase in the blood sugar (13) following the injection of adrenalin. The action of adrenalin, therefore, is not only to cause a higher rate of oxidation but also to mobilize carbohydrate as fuel for this increased combustion; which reaction is primary is not known.

*Diastolic blood pressure.* Throughout the groups there is no constant variation in the diastolic blood pressure. In 19 experiments there was an increase, in 7 there was no change, and in 20 there was a decrease. If the peripheral capillary cross section remains constant when the blood flow is increased the diastolic blood pressure necessarily rises; an unchanged or decreased diastolic with increased blood flow would indicate a compensatory peripheral dilatation. Even a certain proportion of those experiments that showed a slight increase in diastolic blood pressure may likewise have had a slight peripheral dilatation that was not, however, of sufficient degree entirely to compensate for the increased blood flow. A peripheral compensatory dilatation is, therefore, indicated by the data in 27 experiments; it may have occurred in an unknown proportion of the other 19 experiments. Further evidence of peripheral dilatation is shown by the flushing of the skin and increased perspiration, which are physical compensatory factors to accommodate the body to the increased production of heat.

*Systolic blood pressure.* An increase in the systolic blood pressure was noted in all but 4 cases following the adrenalin injection. These four are as follows: One case of adenomas of the thyroid without hyperthyroidism with a basal metabolic rate of +4 per cent; one case of hypopituitarism with a basal metabolic rate of -28 per cent; one case of post-operative myxedema with a basal metabolic rate of -13 per cent; and one case of neurasthenia with a rate of +28 per cent after an injection of thyroxin. In 18 cases the increase was less than 10 per cent; in 17 the increase was between 10 per cent and 20 per cent, and in 7 the increase was 21 per cent or more above the basal. From a study of the individual experiments, of the averages of the various groups of patients, or of the experiments carried out after the patients had received thyroxin, no consistent parallelism can be seen between the percentage increase in the systolic blood pressure following the injection of adrenalin and the degree of over- or under-activity of the thyroid gland.

*Pulse rate.* The pulse rate increased in all but 3 cases: One of these was a case of myxedema in which the basal metabolic rate was -40 per cent; the second, a case of Addison's disease in which the basal



metabolic rate was  $-3$  per cent; and the third, a case of mild exophthalmic goiter in a stage of remission in which the basal metabolic rate was  $+9$  per cent. Ten cases showed less than a 10 per cent increase; all the rest showed a greater response. With an increase in the oxygen consumed and carbon dioxide produced following the injection of adrenalin the circulatory system must transport larger amounts of these and of other substances. This can be accomplished in two ways: *a*, by an increase (15), (16) in the blood flow which can be brought about either by an increase in the number of beats of the heart for each minute, or by an increase in the volume of each beat (or by both, or by a large increase of one with a decrease of the other); and *b*, by a unit volume

TABLE 4

*Summary of effect of subcutaneous injection of adrenalin chlorid on blood pressure, pulse rate, and respiration rate (standard dose of adrenalin chlorid 0.5 cc.)*

NUMBER OF CASES	DIAGNOSIS	AVERAGE B. M. R.	PERCENTAGE INCREASE OVER BASAL	
			Systolic blood pressure	Pulse
		per cent	per cent	per cent
8	Exophthalmic goiter.....	+32	14	18
6	Adenomas of thyroid with hyperthyroidism...	+30	17	11
4	Adenomas of thyroid without hyperthyroidism.....	-1	7	14
2	Colloid goiter.....	+3	3	17
4	Cardiac disease.....	+2	13	25
3	Neurasthenia.....	+4	6	15
1	Malignancy.....	-3	3	21
1	Pulmonary tuberculosis.....	+20	18	6

of blood carrying a greater load. Which of these variable factors will predominate is of course impossible to predict. Furthermore, no consistent relationship can be seen following the injection of adrenalin between the response of the pulse rate and the degree of activity of the thyroid gland.

*Supplementary experiments.* A second series of 29 experiments was carried out in a manner similar to that employed in the preceding series except that the metabolic rates were not determined after the administration of adrenalin. The results are presented in a summarized form in table 4. As in the first series, there is in the various groups an average irregular increase in both pulse rate and systolic blood

pressures. We can discern no parallelism between the changes in pulse rate and blood pressures and the degree of hyperthyroidism that would in any way render the reaction of diagnostic value in such conditions, as has been suggested by Goetsch (17).

#### GENERAL DISCUSSION

These experiments indicate that the injection of adrenalin chlorid produces invariably an increase in the rate of cellular combustion varying between a calorific increase of from 4 per cent to 48 per cent. This increase is accompanied as a rule by an increase in the ventilation rate, respiration rate, number of heart beats for each minute, volume of each beat, greater utilization of the blood-carrying power and peripheral dilatation with an increased systolic and decreased diastolic blood pressure. Not all these compensatory factors necessarily come into play in each instance; as would be expected, various combinations may occur, sometimes one factor, sometimes another factor acting as the major compensation. In individual instances it is impossible to predict the combination, although in perfectly healthy and well-trained persons such as those in the group of normal soldiers studied by Tompkins, Sturgis and Wearn, each compensation factor plays its rôle so well and so easily that there is discernible only the slightest increase of any one factor, while in the case of ill-acting hearts (irritable hearts) the response to extra demands is not smoothly and efficiently accomplished. This is true also in any condition like hyperthyroidism in which the circulatory system is more or less damaged and already severely taxed by its own increased metabolism, and as a result an additional load is not readily borne.

No relationship was found in our experiments between the intensity of the reaction and the degree of hyper- or hypothyroidism. There is no sound physiologic foundation, so far as we can see, for the assumption that the character of the reaction following the injection of adrenalin chlorid is indicative of the activity of the thyroid gland.

The cause of the increased heat production is unknown. The similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk from a carbohydrate plethora naturally directs attention to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. In addition there may be, however, a direct chemical stimulation of cellular combustion. In either case the phenomenon is obviously in harmony with Cannon's (18) "emergency theory" of the action of adrenalin.

## SUMMARY

1. Forty-six experiments are reported on the effect of the subcutaneous injection of adrenalin chlorid on the metabolic rate, pulse rate, and blood pressure of patients suffering from various disorders of the ductless glands.

2. A supplementary series of 27 experiments is added in which a study was made of the effect of the adrenalin injection on the pulse rate, and blood pressure (the basal metabolic rate being known).

3. Adrenalin chlorid (0.5 cc. of 1/1000) injected subcutaneously invariably causes an increase in the metabolic rate. This increase is usually accompanied by an increase in the ventilation rate, respiration rate, number of heart beats each minute, volume of each beat, greater utilization of the blood carrying power and peripheral dilatation with an increased systolic and decreased diastolic blood pressure.

4. No relationship was found between the intensity of the adrenalin reaction and the degree of hyperthyroidism or hypothyroidism.

5. Attention is directed to the similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk from a carbohydrate plethora and to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. It is suggested that in addition there may be a direct stimulation of cellular combustion.

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# THE APPARENT INFLUENCE OF A DIET OF CARBOHYDRATES ON THE PANCREAS REMNANT OF PARTIALLY PANCREATECTOMIZED DOGS

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The experiments here reported were undertaken with the view of determining more definitely whether a carbohydrate diet prevents pancreatic hypertrophy or induces pancreatic degeneration, after partial pancreatectomy. The work was begun in 1916 and the experimental part brought to conclusion in 1917.

The question whether a diet rich in carbohydrates is the etiologic factor in those cases of human diabetes in which impairment of the pancreas cannot be related to known disease processes (cancer, infection, etc.) was put to experimental test in 1912 by Thiroloix and Jacob (1). These investigators reported that partially pancreatectomized dogs, remaining free from diabetes on a diet of meat, develop a fatal diabetes on prolonged carbohydrate feeding. They concluded that the pancreas remnant, adequate to prevent diabetes on a meat diet, is "overstrained" by the prolonged carbohydrate diet. As already noted by Allen (2), these investigators reported no "controls," showing that dogs with equally small pancreas remnants and on a meat diet will not show a similar tendency to gradual atrophy of the pancreas with attendant fatal diabetes. Such controls are necessary, as all workers in this field are agreed that even with the most careful estimate of the mass of pancreas tissue left in situ to just avoid permanent and fatal diabetes, one cannot predict whether the pancreas remnant will undergo hypertrophy so as to render the dog non-diabetic even on a carbohydrate diet, or whether it will atrophy so that the dog becomes permanently diabetic even on a protein diet.

In 1913 Allen (2) reported, in some detail experiments on eight dogs: "None of these observations were as long or as positive as those described by Thiroloix and Jacob. The impression gained, however, is in favor of the correctness of their position, viz., that the condition in

these dogs is aggravated by the carbohydrate diet" (p. 585). "Dogs at the outset diabetic (diabetes levis), yet they recover in spite of the heaviest carbohydrate diet and the most intense glycosuria" (p. 592).

In 1914 Allen (3) reported more positive conclusions, to the effect that dogs with partial pancreatectomy remaining permanently free from diabetes on meat are rendered permanently diabetic on a diet of bread, or bread plus glucose. It is also stated that in such diabetic dogs the island tissue undergoes "hydropic degeneration," similar to that noted by Homans.

In subsequent publications Allen (4) refers frequently to rendering partially depancreatized dogs permanently diabetic by a liberal diet of any of the three classes of foodstuffs (proteins, fats, or carbohydrates). The details of these experiments have, to our knowledge, not been published. It is expressly stated by Allen that fat in the diet brings on diabetes in dogs predisposed to diabetes by partial pancreatectomy, and that the prevention of diabetes in dogs with small pancreas remnants is not accomplished by withholding starches from the diet but by total reduction in the diet amounting to partial starvation. Allen's work on experimental diabetes in dogs has thus been gradually brought in accord with the starvation treatment of human diabetes, as inaugurated by Naunyn, and developed so brilliantly in this country by Allen.

Allen speaks of the hydropic degeneration of island tissue noted after partial pancreatectomy as a *result* rather than the cause of the diabetes—a result of "overstrain" on the endocrine function by a too liberal diet, and support for this "overstrain" hypothesis is sought by analogy in the evil effects of a liberal diet on persons with weakened digestive powers.

#### METHODS

In the preparation of the dogs great care was taken that the following conditions were established: *a*, that the pancreas remnant was connected with the duodenum through the main pancreatic duct, (D. Santorini), and that this duct remained patent; *b*, that each animal following the partial pancreatectomy showed no glycosuria on a liberal protein diet, but showed persistent glycosuria on a carbohydrate diet. Animals meeting these conditions were deemed serviceable for the investigation of this problem.

In the pancreatectomy operation the spleno-gastric and the pyloric portions of the pancreas were freed up to the D. Santorini, ligated off

and removed. Small fragments of pancreas tissue adhering to the duodenum were carefully removed. The pancreas remnant giving the desired glycosuria was assumed to be a fraction of approximately one-seventh of the total pancreas weight. This fraction was determined as follows: A piece of the excised pancreas was trimmed down to match that remaining attached to the duodenum. The weight of this piece added to the weight of all the pancreas tissue removed gives the total pancreas weight. The weight of the fraction of the pancreas remaining on the duodenum was determined by calculation, using the weights thus obtained. If this remnant was in excess of one-seventh of the total weight of the pancreas, the excess was ligated off and removed. Ligation was chosen to prevent hemorrhage of the cut ends, as well as to avoid fat necrosis.

The animals were fed once a day on an excess of one of the diets mentioned below. By an excess of diet is meant a quantity of food so large that some of the food was found in the pan on the following day at the time of the next feeding.

Three types of diets were used, designated as A, B and C.

*Diet A.* This consisted of a mixture of boiled starch, fresh bread and meat. The meat was either lung, liver or skeletal muscle (round steak). This diet contained approximately 60 per cent of carbohydrates.

*Diet B* consisted of a mixture of (equal parts) fresh bread and fresh meat (liver, lung or skeletal muscle). This is a low carbohydrate diet.

*Diet C* consisted of raw or boiled meat (skeletal muscle), depending on the preference of the individual animal. Diet *C* is therefore essentially a protein diet.

Diet *A* could not be fed continually over any long period of time, partly because it seemed to favor constipation, partly because, sooner or later, the dogs refuse to eat it. The two diets, *A* and *B*, were therefore used interchangeably. When the animals refused diet *A*, or alimentary stasis resulted because of constipation, they were given diet *B* and later put back on diet *A*. The diet used for determining the serviceability of the animals at the beginning of the experiment and to determine the degree of glycosuria in the later stages, was diet *B*, because as this contained a greater amount of meat it was ingested in greater quantities.

The animals were supplied with water and meat-free bones ad libitum. Twenty-four-hour samples of urine were collected from the dogs kept in metabolism cages for this purpose. Thymol was added to the urine as a preservative. The urine was analyzed for sugar by Benedict's method.

## RESULTS

*Dog A.* Removed 19.5 grams of the pancreas October 17, 1916. Weight of pancreas remnant estimated 3.5 grams. Weight of dog at time of operation 10.2 kilos.

October 18 to November 6, 1916. Dog on meat diet. No polyuria. A trace of sugar in the urine on October 23, and October 26; urine free from sugar during the rest of the period.

November 7, 1916 to March 7, 1917. Dog on diet B. Slight polyuria (average 250 cc. per day). Sugar in the urine every day (0.21 per cent to 0.90 per cent). Weight of dog on March 7, 8.8 kilos.

March 7 to May 22, 1917. Dog on meat diet. Urine sugar-free. Weight of dog on May 22, 10.6 kilos.

May 23 to 26, 1917. Dog on diet A. No sugar in the urine. Laparotomy performed and 2.2 grams of the pancreas remnant extirpated. The pancreas remnant appeared hypertrophied.

May 27 to June 17, 1917. Dog on meat diet; slight polyuria (about 275 cc. per day) but no sugar in the urine. Weight of dog on June 17, 1917, 10.7 kilos.

June 18 to October 11, 1917. Dog on diet A. Marked polyuria (maximum 2300 cc. per day); gradual loss of weight and persistent glycosuria (2-7 per cent). Weight on October 11, 6.4 kilos. Dog posted. Weight of pancreas remnant, 7.4 grams. Dog extremely emaciated, skin ulcers, conjunctivitis.

This dog died in diabetes, despite the marked hypertrophy of the pancreas remnant. The dog did not tolerate the high carbohydrate diet very well, especially during the month of August, when severe gastro-intestinal disorders (diarrhea, vomiting, anorexia) developed.

*Dog B.* Removed 20.1 grams of the pancreas October 31, 1916. Pancreas remnant estimated at 3.1 grams. Weight of dog 8.5 kilos.

November 1 to 18, 1916. Dog on meat diet. Urine sugar-free.

November 19 to December 4, 1916. Dog on diet B. No polyuria, but persistent glycosuria (1-3 grams per day).

December 5, 1916 to January 8, 1917. Dog on diet A. Polyuria with persistent glycosuria (1-5 grams per day). Weight of dog January 8, 8 kilos.

January 9 to March 7, 1917. Dog on diet B. No polyuria, but slight glycosuria (0.5-1.0 gram per day).

March 8 to May 15, 1917. Dog on meat diet. No glycosuria. Weight of dog on May 15, 10.6 kilos.

May 16 to June 4, 1917. Dog on diet A. Slight polyuria, but no glycosuria. Weight of dog June 4, 8.5 kilos.

Laparotomy performed and 2 grams of the pancreas remnant removed. Remnant appeared hypertrophied.

June 5 to 18, 1917. Dog on meat diet. Slight polyuria, but no glycosuria. Weight of dog June 18, 9.6 kilos.

June 18 to July 22, 1917. Dog on diet A. Marked polyuria (maximum 2100 cc. per day) and persistent glycosuria of gradually increasing intensity (maximum 50 grams per day).

July 23 to 28, 1917. Dog on meat diet. Reduction in the polyuria (to about one-third) and reduction in the glycosuria (to about one-fourth). Weight of dog on July 27, 9.2 kilos.



July 27 to September 30, 1917. *Dog on diet B.* Persistent polyuria and glycosuria, with gradual loss of weight. Death September 30. Body weight 5.6 kilos. Pancreas remnant 5.4 grams. Dog extremely emaciated, with skin ulcers and conjunctivitis.

This dog died in typical diabetes gravis, despite the hypertrophy of the pancreatic remnant. Marked gastro-intestinal disorders developed in July, and were overcome by the meat diet period.

*Dog C.* Removed 15.5 grams pancreas June 14, 1917. Pancreas remnant estimated at 2.3 grams. Weight of dog 7.5 kilos.

June 15 to 21, 1917. *Dog on meat diet.* No glycosuria.

June 22 to August 9, 1917. *Diets A and B.* Polyuria (maximum 1200 cc. per day), glycosuria (maximum 30 grams per day) and progressive emaciation. Death. Weight at death, 5.4 kilos. Pancreas remnant, 2 grams. Pancreatic duct patent. Dog had skin lesions and conjunctivitis.

*Dog D.* Weight 5 kilos; 8 grams of pancreas removed June 5, 1917. Pancreas remnant estimated 1.5 grams. Dog had enlarged thyroid.

June 5 to 24, 1917. *Meat diet.* Trace of sugar in urine, June 6 to 10. Urine sugar-free June 11 to 24.

June 25 to August 6, 1917. *Diet A.* Polyuria (maximum 880 cc. per day) and glycosuria (maximum 35 grams per day), and gradual loss of weight.

August 7 to 9, 1917. Starvation. Urine sugar-free August 8 and 9.

August 10 to September 10, 1917. *Diet A.* Diabetes gravis. Death. Weight of dog, 3.0 kilos. Weight of pancreas remnant, 1.8 grams.

*Dog E.* Weight 5 kilos; 21.2 grams pancreas removed June 4, 1917. Pancreas remnant estimated 3.0 grams.

June 15 to 22, 1917. *Meat diet.* No glycosuria.

June 23 to August 1, 1917. *Diet A.* Polyuria (maximum 1000 cc. per day); glycosuria (maximum 40 grams per day).

August 2 to 7, 1917. Starvation. Reduction of polyuria and glycosuria. Urine sugar-free August 6, 7.

August 8 to 16, 1917. *Diet A.* Gastro-intestinal disorders, polyuria, glycosuria and rapid emaciation. Death in coma. Body weight, 3.0 kilos. Weight of pancreas remnant, 2 grams. Skin ulcers and conjunctivitis.

*Dog F.* Weight 6.8 kilos; 24.3 grams pancreas removed October 27, 1917. Remnant of pancreas estimated 3.7 grams.

October 28 to November 2, 1917. *Diet B.* No polyuria, but glycosuria (maximum 5 grams per day).

November 3 to 16, 1917. *Meat diet.* No glycosuria.

February 17 to 20, 1918. *Diet B.* Glycosuria (maximum 3 grams per day) but no polyuria.

February 21 to June 4, 1918. *Meat diet.* No glycosuria.

June 5 to 9, 1918. *Diet A.* Trace of sugar in urine June 6 and 7, (2.0 grams and 0.3 gram). No glycosuria June 8 and 9. Dog in fine condition. Experiment concluded. Weight of dog, 8.8 kilos. Weight of pancreas remnant, 2.2 grams.

*Dog G.* Weight 6.7 kilos; 18.3 grams pancreas removed March 7, 1918. Pancreas remnant estimated 3.2 grams.

March 8 to 15, 1918. *Meat diet.* No glycosuria.

March 16 to 19, 1918. *Diet A.* Glycosuria (average 3 grams per day) but no polyuria.

March 20 to June 4, 1918. *Meat diet*. No glycosuria.

June 5 to 8, 1918. *Diet A*. No glycosuria. Dog in fine condition. Experiments concluded. Weight of dog, 8.8 kilos. Weight of pancreas remnant, 6.8 grams.

*Dog H*. Weight 6.7 kilos; 23.2 grams pancreas removed February 24, 1918. Pancreas remnant estimated 3.2 grams.

February 25 to 28, 1918. *Meat diet*. No glycosuria.

February 29 to March 3, 1918. *Diet A*. Slight polyuria and glycosuria (average 0.7 gram sugar per day).

March 4 to May 23, 1918. *Meat diet*. No glycosuria.

May 25 to 28, 1918. *Diet A*. No glycosuria. Dog in fine condition. Experiment concluded. Weight of dog, 8.9 kilos. Weight of pancreas remnant, 7.7 grams.

*Dog I*. Weight 7.8 kilos; 22.4 grams pancreas removed October 26, 1917. Pancreas remnant estimated, 3.1 grams.

October 27 to 31, 1917. *Meat diet*. No glycosuria.

November 1 to 4, 1917. *Diet B*. Slight glycosuria (2 grams per day).

November 4 to December 21, 1917. *Meat diet*. No glycosuria.

December 22 to 24, 1917. *Diet B*. Slight glycosuria (1.6 gram per day).

December 25, 1917 to February 5, 1918. *Meat diet*. No glycosuria. Dog died by accident. Body weight, 6.4 kilos. Weight of pancreas remnant, 3.3 grams.

#### COMMENTS AND CONCLUSIONS

1. In general, our results support the view that a liberal carbohydrate diet tends to change diabetes levis into diabetes gravis, after partial pancreatectomy in dogs. But our experiments do not constitute a clear demonstration of this thesis, in fact they are not much more conclusive than the experiments reported earlier by Thiroloix, and by Allen.

It seems to us that the question can be settled by the statistical method only, using a great many animals for the work, because of the fact that one cannot predict the metabolic course of any one animal deprived of approximately seven-eighths of the pancreas. When a considerably smaller pancreas remnant is left the dog passes into fatal diabetes irrespective of the diet; if the pancreas remnant is somewhat larger no amount of forced carbohydrate feeding induces diabetes gravis. And since the functional efficiency of the pancreas appears to vary in different dogs, the estimation, by weight, of the optimum pancreas remnant for the solution of this question involves a large and variable error.

The analogy from an impaired digestion is not to the point. It is true that ingesting certain foods, or any food in certain amounts, may aggravate the untoward condition of an alimentary tract whose digestive juices and motor power are much below normal, but this is not due to any "overstrain" of the digestive glands, or of the gastro-intestinal

motor mechanisms. It is due to stasis, bacterial fermentation and putrefaction, and to abnormal reflexes evoked from an hyperirritable gut.

2. The view of organ impairment as a direct result of physiological "overstrain" is simple, is readily strengthened by analogy, and, viewed with little or no critical analysis, appears so reasonable that it is being used as sufficient explanation of results in physiology and medicine to a greater extent than the facts warrant. It is true that excessive stimulation of the eye or the ear leads to degeneration in the retinal and cochlear elements. But it is equally true that surgical removal of a large percentage of such glands as the kidney, the thyroid, etc., causes hypertrophy, not atrophy of the gland remnants, unless the gland remnant is so small that conditions are produced in the body which are inimical to growth and repair in general. The facts in regard to the thyroid gland are the most striking in this connection. When one attempts to completely extirpate the thyroid in young animals (rabbits, chickens), it frequently happens that a few thyroid cells (less than  $\frac{1}{10}$  part of the gland) are left *in situ*. These few cells invariably multiply up to a point where the gland substance meets the internal needs of the animal. We must assume that when a few thyroid cells are called on to function for the entire thyroid gland, these few cells are being greatly over-stimulated, as compared to the normal. Nevertheless the result is hypertrophy, not atrophy.

3. When the functional capacity of the pancreas remnant is too small to prevent diabetes, the pancreas remnant undergoes practically no hypertrophy. On this point our results are in agreement with those of previous workers. This failure to hypertrophy may be due to the unfavorable influence on growth in general of the diabetic condition, rather than to specific "overstrain" of the pancreas remnant.

4. The favorable effects, at least in many cases, of partial starvation in the management of diabetic patients cannot at present be ascribed, in whole or in part, to relieving an impaired (endocrine) pancreas from the "overstrain" of ordinary diets, in view of the indications from human and comparative physiology that starvation, within limits, tends to rejuvenate tissues in general.

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# THE COMPARATIVE PERFORMANCE OF MUSCLES SUBJECTED TO RHYTHMIC AND ARHYTHMIC STIMULATION

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In making records of simple muscle contractions in series to observe the oncoming of fatigue and the final failure of response, it has been customary to send in the stimuli at uniform intervals. It is of interest to find out whether muscles thus treated give a better or a poorer total performance than symmetrical muscles stimulated an equal number of times per minute but with quite irregular spacing of the contractions. If work done is to be our standard, the intervals must in no case be so short as to cause marked summation.

In the present study gastrocnemii of frogs were stimulated directly. The primary current was made and broken as brass brushes trailed on the periphery of brass discs from which segments had been cut. The discs were revolved by a motor, make shocks were excluded by an appropriate device and breaks delivered to the muscle. In one set of discs the divisions of the rim were equal; in the companion set they numbered the same but were of unequal length. In a typical experiment the gastrocnemius from the right leg might be stimulated rhythmically and its fellow from the left leg arhythmically. Graphic records (see fig. 1) were made and the comparative accomplishment measured with a work-adder. The frequency of stimulation varied from 14 to 56 per minute.

A tabulation of fifty experiments, in each of which two symmetrical muscles were matched, shows no significant difference between the results attained in the two types of trial. In solitary cases, differences in the figures for the work done approached 25 per cent but one variety of performance was favored about as often as the other. Generally the muscles compared registered amounts of work which differed by less than 10 per cent. The average of all the experiments gave the following close correspondence. Work done by 50 muscles stimulated at regular intervals, 4255 gram centimeters; by 50 muscles stimulated at irregular intervals, 4276 gram centimeters.

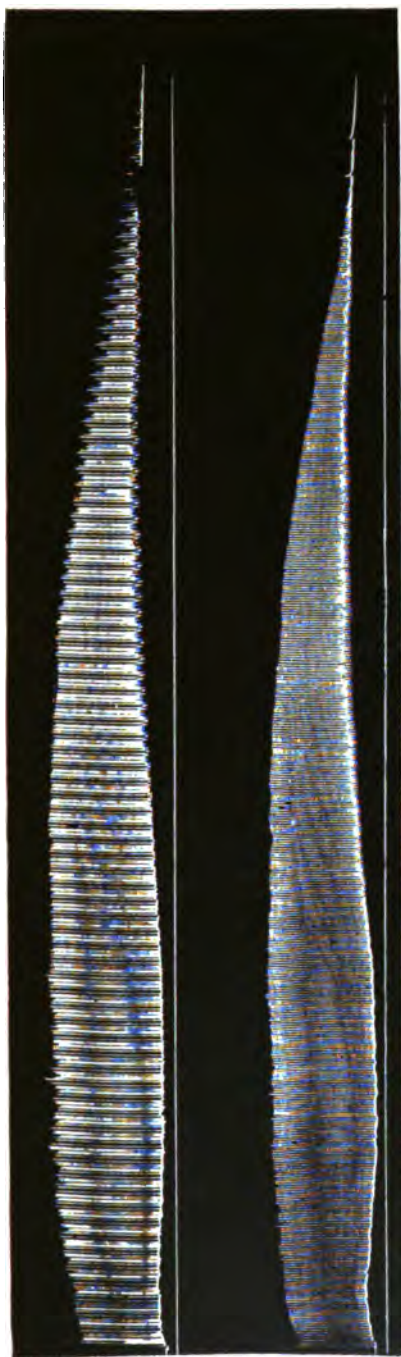


Fig. 1. Experiment 17. The upper tracing shows the contractions of the left gastrocnemius muscle of a frog stimulated arhythmically; the lower tracing, those of the right stimulated rhythmically.

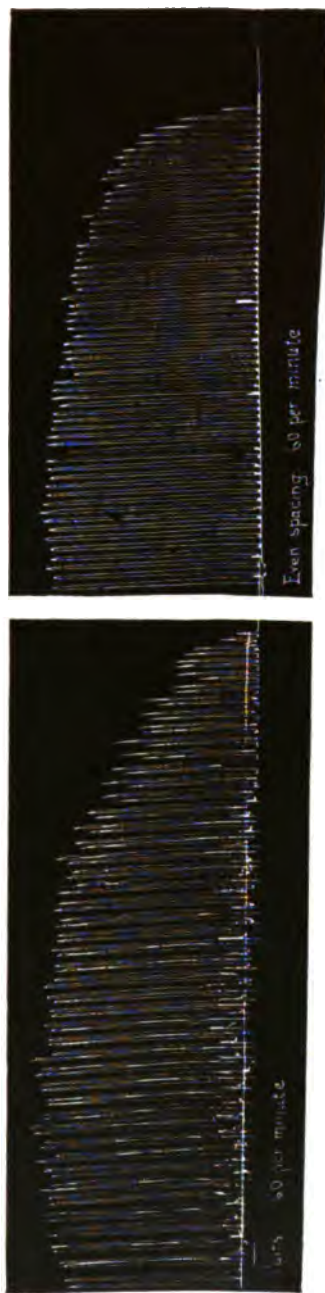


Fig. 2. Illustrating the superiority of an ergograph record with paired contractions. In this case the performance exceeded that with the simple rhythm by about 30 per cent.

The equality noted above naturally raises the question of voluntary contractions. Can one do more work with a weight ergograph when the efforts are made at rigidly uniform intervals or when the intervening periods are alternately long and short? The inquiry has possible bearings upon industrial fatigue. In a series of contractions we may make the interval 2 seconds throughout or it may be alternately 3 seconds and 1 second. Thirty contractions in a minute will be recorded with either procedure. We may group them in other and less simple ways, making three or four efforts in quick succession and thus earning a relatively long rest.

The cue for making the contractions was furnished in our experiments by the appearance from behind a screen of vertical lines borne upon the surface of a revolving drum. These lines presented themselves singly, in pairs, in threes or in fours, as desired, but in every case the number for a revolution of the drum was the same. The frequency was about 50 per minute. The weight, lifted by the middle finger of the right hand with the strap on the distal phalanx, was about 2 kgm. Sometimes a work-adder was connected with the ergograph but the total number of lifts accomplished proved to be a satisfactory criterion.

It soon became obvious that more work can be done when long and short intervals are alternated than when the spacing of the efforts is uniform. The average performance for 12 trials with the contractions in pairs exceeds the average for 16 trials with the contractions evenly spaced by 41 per cent. When the contractions are thrown into groups of three or four with rest periods correspondingly lengthened there is a slight additional gain (fig. 2).

The advantage of such an ordering of work over the simple rhythm appears to be due mainly to a single factor. This is not, as might be anticipated, the longer rest period which keeps recurring; it is, paradoxically, the *shorter* one. The facts seem to be as follows: When a man is compelled to execute a contraction quickly in order to be ready for the next one in its turn *he actually draws less upon his muscular resources than he would if he "took his time."* There is an element in this type of experiment which was not present when the frog muscle was stimulated by single shocks. Voluntary contractions are tetanic in character and of variable duration. The economy of the short, sharp contraction is readily demonstrated. Given 2 seconds in which to contract and relax, one may devote a second to the up-stroke and use the other for the return or it may be possible to get both into the first second and have the remaining half of the time for complete

repose. When the results of these two methods were compared it was found that the first plan led to complete fatigue after 144 contractions while the second did not exhaust the subject in 300 lifts.

It is reasonable to expect a postponement of fatigue with reduced periods of tetanization. If our reasoning is correct one might hope to cultivate a snappy way of executing all contractions which should equalize his rhythmic and arhythmic records. But it is hard to provide an incentive so compelling as the knowledge that a given movement must be finished in time to begin another. In all ergographic studies it must be important to attend to the form of the separate curves as they appear upon a rapidly moving surface. This matter has generally been ignored. There are probably individual peculiarities and it is evident in our own cases that the instinctive mode of raising the weight is not the best attainable from the standpoint of economy. To be hurried to a certain degree proves to be distinctly advantageous.

## RENAL ACTIVITY AND THE ACID BASE EQUILIBRIUM

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In order to determine whether changes in the acid base equilibrium of the body influence renal activity, the urea excreting capacity of the kidney was measured after the administration of acid and alkaline sodium phosphate salts. These substances are particularly suitable for this purpose since the same work is imposed on the kidney in the excretion of the phosphate radical, and the only essential difference between them is that the acid phosphate leads to a shift in the neutrality equilibrium toward the acid side, while the alkaline phosphate has the opposite effect. Control experiments can be carried out with a mixture of acid and alkaline phosphate of neutral reaction. The degree of change in the acid base balance is indicated by the extent of the decrease in the carbon dioxide combining power of the plasma after acid phosphate, and the amount of increase which follows the administration of alkaline phosphate.

The ratio between the urea content of the urine and of the blood was taken as the measure of the urea excreting activity of the kidney. The methods and the technique employed by Addis, Barnett and Shevky (1) were followed, involving the collection of four successive samples of urine and of blood over a period of five hours. Five grams of urea were given with the phosphate in order that variations in the ratio arising from other causes than alterations in the neutrality equilibrium should as far as possible be diminished (2). The carbon dioxide combining power of the plasma and the H-ion concentration of the urine were determined during each of the four periods of the test. Van Slyke, Stillman and Cullen's (3) titration method was used for the plasma carbon dioxide, after a parallel series of experiments had shown that in rabbit's plasma essentially the same results were obtained by this method as by the direct evacuation and measurement of the carbon dioxide. Henderson and Palmer's method (4) was used in determining the acidity or alkalinity of the urine, except that 1 cc. instead of 10 cc. of urine was taken.



It was intended that the control and the acid and alkaline experiments should be carried through on a group of twenty rabbits, but five of the twenty died before all the experiments had been completed. On each of the remaining fifteen animals all the experiments described in this and in the succeeding paper were performed and since the comparison of the effects of the various substances given is facilitated by dealing only with this uniform group of fifteen, the partial results obtained on the other five are excluded.

In order that the increase of work thrown on the kidney in the excretion of phosphate should be as far as possible the same, the amounts of neutral, acid and alkaline phosphates given were adjusted so that they contained an equal number of phosphate radicals. It is of course true that this fact does not allow us to assume that there was an equal

TABLE 1

*Effect of neutral, acid and alkaline phosphate on the ratio:  $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$*   
*after the administration of urea*  
*Averages of all periods from experiments on a group of fifteen rabbits*

SUBSTANCE GIVEN	PLASMA CO <sub>2</sub>	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
				<i>mgm. per hour</i>	<i>mgm. per 100 cc.</i>	
Neutral phosphate.....	52	22	6.7	499	161	3.09
Acid phosphate.....	39	15	5.9	299	158	1.86
Alkaline phosphate.....	56	20	6.8	479	157	3.04

absorption of phosphate from the intestine. But Underhill and Bogert (5) were unable to distinguish any difference between the degree of increase in phosphate excretion in the urine produced by the subcutaneous injections into rabbits of acid as opposed to alkaline sodium phosphate. Phosphate estimations in the urine in our experiments would not have been decisive, since we have no means of distinguishing between changes in excretion due to variations in the rate of absorption and those due to variations in the phosphate excreting activity of the kidney. Only the subcutaneous or intravenous injection of the salts would have removed all doubt in regard to this point, but such operative procedures introduce so many variable factors, that the interpretation of the effect on renal function is apt to be obscured.

For the control experiments a mixture of 0.5 gram NaH<sub>2</sub>PO<sub>4</sub>, 4H<sub>2</sub>O and 2.32 grams Na<sub>2</sub>HPO<sub>4</sub>, 2H<sub>2</sub>O was made. The pH of this mixture

in solution was 7.48. In the acid experiments 3 grams of  $\text{NaH}_2\text{PO}_4 \cdot 4\text{H}_2\text{O}$  were given and in the alkaline 2.81 grams of  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ . In all cases 5 grams of urea were added to these salts and the whole diluted to 35 cc. with water and administered through a stomach tube just before the bladder was emptied by catheter at the commencement of the collection of the first hour's urine. Although these are large amounts of phosphate no effect on intestinal excretion was observed.

TABLE 2

*Effect of neutral, acid and alkaline phosphate on the ratio:  $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$   
after the administration of urea  
Averages of separate periods*

PERIOD	PLASMA $\text{CO}_2$	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
1. Neutral phosphate. Control experiments						
	vol. per 100 cc.	cc.		mgm. per hour	mgm. per 100 cc.	
1	48	22	7.2	388	144	2.68
2	52	24	6.6	562	171	3.26
3	55	17	6.8	571	168	3.41
4	54	23	6.3	474	160	2.99
2. Acid phosphate						
1	41	9	6.3	160	116	1.39
2	38	10	5.9	276	156	1.76
3	37	15	5.7	404	181	2.27
4	39	24	5.6	356	177	2.02
3. Alkaline phosphate						
1	50	17	7.3	341	131	2.60
2	56	21	6.8	517	168	3.07
3	60	18	6.7	580	163	3.55
4	59	22	6.5	480	157	3.07

The average results obtained by combining the four observations made on each of the fifteen rabbits are given in table 1.

A more detailed conception of the effect of acid and alkaline phosphate can be obtained from the average four periods shown in table 2 and charted in figure 1. The data from which these averages were obtained are given in tables 3, 4 and 5.

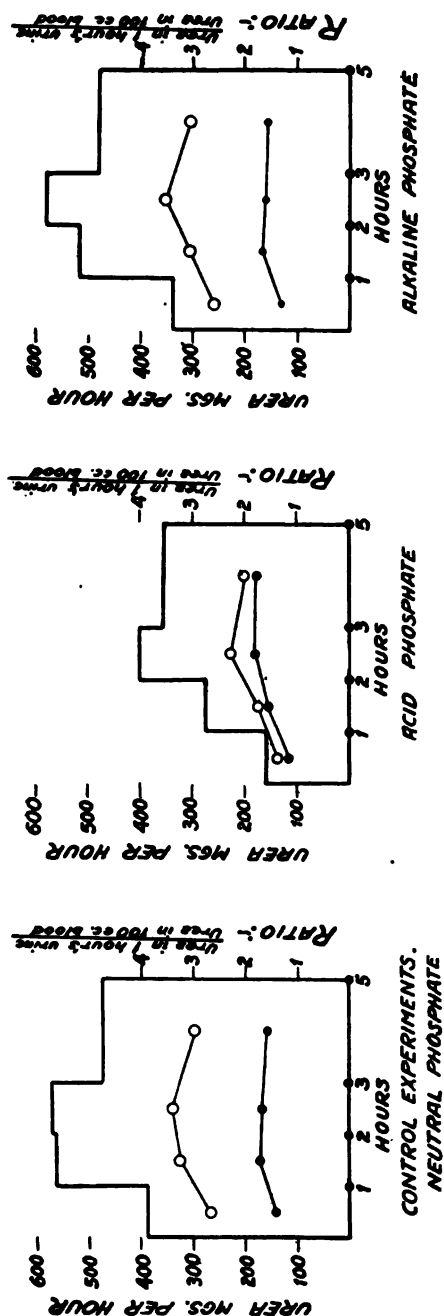


Fig. 1. Showing the decrease in the urea excreting activity of the kidney produced by acid sodium phosphate. The ratio between the urea in one hour's urine and the urea in 100 cc. of blood is shown by circles joined by lines. The value is given on the scale at the right of the charts. The hourly rate of urea excretion is represented by the blocked areas. The blood urea concentration is shown by dots joined by lines. The values for both the rate and the blood concentration are given by the scale on the ordinates at the left of the charts.

These figures show that the shift in the neutrality equilibrium toward the acid side indicated by a reduction in the carbon dioxide combining capacity of the plasma from 52.4 to 38.7 volumes per cent is accompanied by a distinct decrease in the urea excreting activity of the

TABLE 3  
*Control experiments (neutral phosphate)*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period I						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	50.5	25.0	7.4	340	129	2.63
2	52.3	16.0	7.4	425	165	2.57
3	47.0	14.0	7.5	240	117	2.05
4	50.2	19.0	7.5	400	150	2.67
5	48.0	29.0	7.4	447	126	3.55
9	54.5	20.5	7.6	285	123	2.32
10		22.5	7.0	531	192	2.77
11	46.8	20.0	6.3	266	153	1.74
12	49.3	38.0	7.2	570	165	3.45
13	44.0	21.0	7.0	531	150	3.54
14	54.6	47.0	8.0	301	129	2.33
15	40.4	15.0	6.9	374	114	3.37
16	45.8	26.0	7.4	494	162	3.05
19	45.5	15.0	7.4	375	177	2.12
20	43.5	8.0	6.3	242	111	2.18
Period II						
1	52.5	24.5	6.3	540	156	3.46
2	51.8	21.0	6.7	598	195	3.07
3	49.4	19.0	6.9	390	150	2.60
4	53.2	18.0	7.4	475	189	2.51
5	55.1	28.0	6.3	544	159	3.42
9	51.8	15.0	7.4	285	150	1.90
10	51.9	24.0	6.3	730	213	3.43
11	53.8	29.0	6.0	564	186	3.03
12	53.4	27.0	6.3	631	180	3.51
13	56.0	23.0	6.3	740	174	4.25
14	57.0	28.0	7.0	460	153	3.01
15	50.3	18.0	6.3	603	165	3.65
16	54.3	38.0	7.4	621	162	3.83
19	50.2	16.0	6.7	620	186	3.33
20	44.8	24.0	6.3	634	168	3.78

TABLE 3—*Concluded*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period III						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	55.0	17.0	6.3	541	147	3.68
2	50.0	16.0	6.3	659	180	3.66
3	51.6	20.0	6.3	520	150	3.33
4	56.4	18.0	7.0	554	190	2.83
5	60.2	16.0	6.3	494	150	3.29
9	50.5	18.0	7.0	475	168	2.83
10	51.2	15.0	6.3	681	192	3.55
11	55.5	24.0	6.0	546	177	3.09
12	59.1	14.0	6.3	518	159	3.26
13	57.0	20.0	6.3	749	168	4.46
14	57.0	21.0	6.0	530	171	3.10
15	48.0	11.0	6.0	508	153	3.32
16	58.5	15.0	7.0	529	162	3.27
19	54.7	13.0	6.3	599	171	3.50
20	56.5	22.5	5.9	660	168	3.93
Period IV						
1	55.0	22.0	6.0	413	135	3.06
2	49.2	19.0	6.3	470	177	2.65
3	56.0	26.0	6.3	453	156	2.90
4	60.8	26.0	6.7	505	192	2.63
5	59.0	19.0	6.0	359	153	2.35
9	56.0	26.0	7.0	471	150	3.14
10	51.5	20.0	6.3	509	183	2.78
11	54.6	38.0	6.0	460	171	2.69
12	54.8	20.0	6.3	425	141	3.01
13	56.8	23.0	6.3	585	141	3.98
14	50.2	35.0	6.3	445	174	2.86
15	45.8	16.5	6.0	504	153	3.29
16	54.3	17.5	6.7	469	147	3.19
19	54.7	18.0	6.3	537	165	3.25
20	56.9	21.0	6.0	510	150	3.40

kidney. The urea concentration of the blood is almost the same yet 499 mgm. of urea per hour are excreted after neutral phosphate, and only 299 mgm. per hour after acid phosphate. The average ratio between the urea content of the urine and the blood is thus reduced from 3.08 to 1.86. This is a difference which is much greater than any

which could have arisen by chance. In the four periods the ratios after acid phosphate are less than the ratios in the control experiments by amounts which are respectively 9.9, 11.2, 7.5 and 8.7 times greater than the "probable differences between the averages" (6).

TABLE 4  
*Experiments with acid phosphate*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period I						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	44.6	8.5	6.0	134	108	1.24
2	36.6	11.0	6.3	296	129	2.29
3	42.6	8.0	6.3	176	102	1.72
4	47.3	8.5	6.0	135	120	1.13
5	42.0	16.0	6.0	263	105	2.50
9	42.5	8.0	6.7	120	135	0.89
10	44.5	6.0	7.4	86	105	0.84
11	33.1	7.0	5.0	96	75	1.28
12	36.6	9.0	6.0	250	138	1.81
13	51.5	12.0	7.0	234	150	1.55
14	40.4	9.0	6.0	134	111	1.21
15	31.7	6.0	5.7	114	93	1.23
16	37.8	8.5	6.3	180	132	1.36
19	41.1	4.5	7.4	71	126	0.56
20	44.6	8.0	6.0	118	105	1.13
Period II						
1	30.0	8.0	5.7	261	153	1.71
2	37.2	12.0	6.3	405	156	2.60
3	38.1	9.0	6.3	237	132	1.79
4	32.7	8.5	6.0	210	165	1.27
5	41.0	11.0	6.0	299	141	2.12
9	40.3	6.5	6.3	227	177	1.28
10	40.3	13.0	6.3	279	147	1.90
11	32.7	6.0	4.7	120	126	0.95
12	35.8	13.0	5.7	428	171	2.50
13	49.1	14.0	6.0	370	189	1.96
14	38.9	4.0	6.0	177	174	1.02
15	31.3	7.0	6.0	245	135	1.81
16	40.0	20.0	6.0	310	162	1.92
19	37.5	9.0	6.0	204	162	1.26
20	44.6	9.0	5.5	370	156	2.37

TABLE 4—*Concluded*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period III						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	35.4	15.0	5.7	299	171	1.75
2	37.4	16.5	5.4	564	162	3.48
3	25.3	9.5	6.3	256	144	1.77
4	34.9	15.0	6.0	198	177	1.12
5	42.5	17.0	5.5	425	153	2.78
9	38.6	9.0	6.3	305	237	1.29
10	36.6	15.0	6.2	417	195	2.14
11	34.5	26.0	4.7	593	162	3.66
12	35.8	17.0	5.3	535	189	2.83
13	47.0	18.0	6.0	551	201	2.74
14	42.5	9.0	5.2	358	216	1.66
15	30.8	9.0	5.7	331	150	2.21
16	36.4	22.5	6.0	443	216	2.05
19	36.6	12.5	5.7	282	183	1.54
20	40.2	15.0	5.3	505	165	3.06
Period IV						
1	37.5	29.0	5.5	205	189	1.09
2	37.9	24.5	5.6	456	159	2.86
3	39.4	30.0	5.7	349	147	2.38
4	37.4	14.0	5.7	187	174	1.07
5	41.5	44.0	5.0	390	165	2.36
9	37.2	16.0	6.0	337	195	1.73
10	44.8	28.0	6.0	318	180	1.77
11	35.8	33.0	4.7	424	183	2.32
12	37.0	16.0	5.7	284	174	1.63
13	46.0	23.0	6.0	480	186	2.58
14	42.0	23.0	5.7	455	213	2.14
15	35.7	21.5	5.8	346	161	2.10
16	38.9	14.0	5.7	396	180	2.20
19	37.5	26.5	5.5	312	180	1.73
20	42.5	22.0	5.3	398	165	2.41

On the other hand it is equally plain from the results shown in tables 1 and 2 that the alkaline phosphate had no appreciable effect on the urea excreting activity of the kidney. The slight and irregular differences between the ratios of the control and alkaline phosphate experiments might well be due to chance. But it will be noted that the car-

bon dioxide content of the plasma is only slightly increased. On account of the lesser solubility of the alkaline phosphate it was impossible to give a quantity which would markedly change the balance toward the alkaline side. All that can be said is that an increase in

TABLE 5  
*Experiments with alkaline phosphate*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period I						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	51.5	26.0	7.0	445	150	2.97
2	56.5	15.5	8.0	424	150	2.83
3	52.3	14.0	7.7	288	132	2.18
4	58.5	22.5	8.0	339	146	2.35
5	47.0	13.5	7.0	367	126	2.91
9	50.0	16.0	7.7	350	168	2.12
10	55.9	27.0	7.7	520	156	3.33
11	51.8	7.0	5.7	165	60	2.75
12	55.8	20.0	7.4	409	166	2.84
13	44.8	18.0	6.7	400	120	3.33
14	53.6	16.0	7.4	231	111	2.08
15	45.0	18.0	7.0	380	165	2.30
16	48.2	18.0	7.4	180	69	2.61
19	41.5	13.5	7.4	350	147	2.38
20	40.3	11.0	6.7	268	132	2.03
Period II						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	59.0	27.5	5.7	566	177	3.20
2	60.9	23.0	8.0	495	165	3.00
3	59.0	22.5	7.0	504	159	3.17
4	58.5	23.0	7.4	410	165	2.49
5	50.5	16.0	6.0	504	165	3.05
9	58.1	18.0	7.0	509	177	2.88
10	55.9	20.5	7.7	455	183	2.49
11	54.5	18.0	6.3	580	177	3.28
12	61.8	25.5	7.0	690	168	4.11
13	60.2	20.0	6.9	670	156	4.29
14	49.2	9.5	7.0	350	141	2.48
15	50.0	18.0	6.3	510	183	2.79
16	53.8	33.0	7.0	393	162	2.42
19	53.6	19.5	6.7	510	177	2.88
20	50.5	20.0	6.3	608	171	3.55



TABLE 5—*Concluded*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period III						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	63.6	21.0	6.7	631	156	4.05
2	67.0	19.0	7.4	566	159	3.56
3	58.1	16.0	7.0	508	153	3.32
4	58.1	29.0	7.4	555	159	3.49
5	56.4	14.5	5.7	580	156	3.72
9	59.0	15.0	7.0	520	165	3.15
10	56.7	19.0	7.4	580	183	3.17
11	60.2	19.0	6.3	705	183	3.85
12	67.0	17.0	7.0	720	177	4.07
13	66.2	16.0	6.9	710	159	4.46
14	48.4	14.0	6.7	420	150	2.80
15	53.3	15.5	6.3	573	147	3.90
16	60.2	15.0	6.7	598	177	3.38
19	60.5	20.0	6.3	472	168	2.81
20	61.2	14.5	6.3	555	159	3.49
Period IV						
1	64.3	20.0	6.3	405	150	2.70
2	62.5	23.0	7.0	530	150	3.53
3	58.1	23.0	6.7	455	156	2.92
4	67.2	25.0	7.0	497	171	2.91
5	62.5	18.0	6.0	530	153	3.46
9	55.0	18.0	6.7	454	153	2.97
10	48.3	24.0	7.0	432	174	2.48
11	65.7	28.0	6.3	585	159	3.68
12	65.8	20.0	6.3	436	141	3.09
13	69.2	24.0	6.9	540	141	3.83
14	48.0	33.5	6.3	455	150	3.03
15	46.0	17.5	6.3	440	162	2.72
16	64.6	24.0	6.3	570	183	3.12
19	57.0	19.0	6.3	389	153	2.54
20	56.6	19.0	6.0	475	153	3.11

carbon dioxide combining power of from 52.4 to 56.3 volumes per cent produced by the administration of alkaline phosphate has no definite influence on renal activity.

By giving sodium bicarbonate it is possible to produce a change toward the alkaline side as marked as the change toward acidity,

induced by acid phosphate. The conditions of the experiment are not so uniform since the kidney is under different conditions in excreting an excess of neutral phosphate and an excess of sodium bicarbonate, but we believe the experiment is of value in judging the probable general effect of a pronounced increase in the potential alkalinity of the plasma. The same group of fifteen rabbits was given 3 grams  $\text{NaHCO}_3$  dissolved with 5 grams of urea in 35 cc. of water. The average results

TABLE 6

Effect of sodium bicarbonate on the ratio:  $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$  after the administration of urea  
Averages of all periods from experiments on a group of fifteen rabbits

PLASMA $\text{CO}_2$	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
vol. per 100 cc.	cc.		mgm. per hour	mgm. per 100 cc.	
73	26	8.4	461	159	2.91

TABLE 7

Effect of sodium bicarbonate on the ratio:  $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$  after the administration of urea  
Averages of separate periods

PERIOD	PLASMA $\text{CO}_2$	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
	vol. per 100 cc.	cc.		mgm. per hour	mgm. per 100 cc.	
1	67	28	8.0	390	144	2.73
2	73	28	8.4	482	164	2.96
3	74	23	8.6	525	167	3.12
4	76	26	8.6	448	161	2.82

are compared with the neutral phosphate controls in tables 6 and 7 and charted in figure 2. The details are given in table 8.

Under the conditions of this experiment a marked shift toward alkalinity is associated with a slight decrease in the urea excreting activity of the kidney. With almost the same urea concentration of the blood 499 mgm. of urea per hour are excreted after neutral phosphate and 461 mgm. per hour after sodium bicarbonate, a ratio of 3.1 is reduced to 2.9. In the first period the ratios are practically identical, but in the succeeding periods when the greatest effect on the carbon dioxide

combining power of the plasma is produced the ratios after bicarbonate are less than the corresponding control ratios by amounts which are respectively 2.0, 2.5 and 1.7 times larger than the probable differences. Although the decrease in none of the periods separately is enough to exclude chance, yet taken together they make it improbable that the divergence is not due to a specific difference in the effects of the neutral phosphate and the bicarbonate. It cannot with certainty be said that this difference is due to the fact that the one is neutral and that the other is alkaline, but the demonstration that acid phosphate depresses renal function on account of its acidity lends support to that interpretation.

I am indebted to Dr. T. Addis and Mr. A. E. Shevky for suggesting work on this subject.

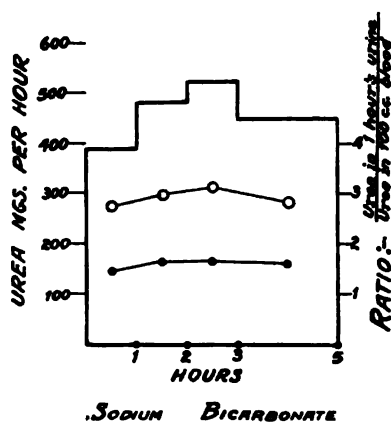


Fig. 2. Showing the effect of sodium bicarbonate.

#### CONCLUSIONS

1. The urea excreting activity of the kidney under strain was measured after the administration of a mixture of acid and alkaline phosphate of neutral reaction, and also after the administration of an amount of acid phosphate containing the same amount of phosphorus. A distinct decrease in function was observed after acid phosphate. Since the only essential difference in the conditions of these experiments lay in the fact that after the neutral phosphate mixture the acid base equilibrium remained unchanged, whereas after acid phosphate there was a shift toward the acid side, it is concluded that the decrease in the alkalinity of the plasma induced by an increase in the amount of

acid phosphate within the body, decreases the urea excreting activity of the kidney.

2. The administration of an amount of alkaline phosphate containing an amount of phosphorus equivalent to that given in the experi-

TABLE 8  
*Experiments with sodium bicarbonate*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period I						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	69.7	27.0	7.4	430	168	2.56
2	64.7	30.0	8.7	347	126	2.76
3	77.3	42.0	8.7	350	144	2.43
4	69.4	32.0	8.0	483	126	3.84
5	64.9	46.0	8.0	560	135	4.15
9	78.0	25.0	8.7	359	177	2.03
10	74.0	16.0	8.7	350	183	1.91
11	47.0	7.5	7.2	104	72	1.44
12	58.0	19.0	8.4	365	126	2.90
13	85.0	16.5	8.0	467	129	3.62
14	60.2	53.0	7.6	518	186	2.78
15	64.8	41.0	8.0	492	171	2.88
16	60.2	30.0	8.0	394	144	2.74
19	64.0	14.0	7.4	293	144	2.04
20	71.0	16.0	7.2	345	123	2.80
Period II						
1	72.0	23.0	8.0	494	177	2.79
2	85.1	44.0	8.7	473	147	3.22
3	72.9	28.0	8.7	489	171	2.86
4	62.7	18.0	8.7	267	141	1.89
5	69.1	33.0	8.0	655	153	4.28
9	84.0	23.0	8.7	465	198	2.35
10	87.1	20.0	8.7	473	192	2.46
11	58.2	12.0	8.5	396	123	3.22
12	78.2	36.0	8.4	537	180	2.98
13	69.0	36.0	8.4	465	132	3.52
14	70.8	28.0	8.7	465	186	2.50
15	78.1	33.0	8.4	629	186	3.38
16	71.5	49.5	8.4	455	162	2.81
19	68.0	16.5	8.4	450	159	2.83
20	73.8	20.5	7.4	513	156	3.29

TABLE 8—*Concluded*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period III						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	70.0	21.0	8.7	600	189	3.18
2	64.7	30.0	8.7	347	126	2.76
3	72.9	28.0	8.7	489	171	2.86
4	69.4	15.0	8.7	399	141	2.83
5	78.2	16.0	8.7	589	156	2.79
9	80.5	22.0	8.7	558	180	3.10
10	86.0	15.0	8.7	467	204	2.29
11	62.0	18.0	8.7	416	114	3.65
12	79.0	20.0	8.4	585	171	3.42
13	75.0	27.0	8.4	543	150	3.62
14	73.0	21.0	8.7	533	207	2.58
15	74.5	15.5	8.7	505	174	2.90
16	79.0	27.0	8.7	646	189	3.42
19	67.1	16.5	8.7	526	168	3.13
20	82.5	53.5	8.0	674	159	4.24
Period IV						
1	71.5	29.5	8.0	498	168	2.96
2	79.3	21.0	8.7	353	141	2.51
3	75.0	30.0	8.7	435	156	2.79
4	73.9	23.0	8.7	342	129	2.65
5	72.1	15.0	8.2	439	144	3.05
9	74.0	22.5	8.7	419	183	2.29
10	79.0	23.0	8.7	486	198	2.46
11	62.0	18.0	8.7	416	114	3.65
12	80.5	27.5	8.7	501	153	3.27
13	72.8	30.0	8.7	530	160	3.31
14	82.1	23.0	8.7	404	195	2.07
15	78.5	19.0	8.7	420	162	2.59
16	80.5	32.0	8.7	485	180	2.70
19	71.2	18.0	8.7	417	168	2.48
20	80.5	54.0	8.4	580	168	3.45

ments with neutral and acid phosphates, only slightly increased the alkalinity of the plasma and had no appreciable effect on renal function.

3. The administration of amounts of sodium bicarbonate which markedly increased the alkalinity of the plasma was accompanied by a slight decrease in the urea excreting activity of the kidney.

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## THE UREA EXCRETING ACTIVITY OF THE KIDNEY AND PHOSPHATE EXCRETION

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The quantity of 5 grams of urea given in the experiments on rabbits detailed in the preceding paper increases the blood urea concentration to an abnormally high level. The kidney responds with a rate of work in urea excretion considerably higher than any required under physiological conditions. And when, as in the above experiments, there is at the same time a pronounced increase in the amount of phosphates, another strain, also exceeding in extent any previously experienced, is suddenly thrown on the excretory capacity of the kidneys. Under these conditions it is of interest to determine whether the urea excreting capacity is altered on account of the simultaneous increase in phosphate elimination.

It has been shown (1) that a moderate increase in the urea content of the blood is followed by a proportionally greater increase in the urea content of the urine. Evidence has been given that this increase in excretory activity is brought about through the intervention of extra-renal factors under the control of the nervous system, which are able to augment the effectiveness of the anatomical mechanism concerned with urea excretion (2). But there is a limit to the action of these compensatory factors, for with a continued increase in blood urea concentration, the ratio between the urea content of the urine and of the blood increases more and more slowly. After a level of 150 mgm. of urea per 100 cc. of blood is reached, there is very little change in the ratio, and there is a definite decrease at concentrations above 225 mgm. per 100 cc. At the point at which the ratio ceases to increase, the rate of urea excretion has attained its maximum; any call for further work in the form of a still greater increase in the urea content of the blood is unavailing and leads only to a decrease in the ratio. Under these conditions the kidney becomes insufficient because the structural mechanism underlying urea excretion has already attained its fullest capacity for work (3).

In this paper the ratio after 5 grams of urea is compared with the ratio after 5 grams of urea given with a neutral mixture of acid and alkaline sodium phosphate. In both experiments the blood urea concentration rose after the first period to levels between 160 and 175

TABLE 1

*Comparison of the effect of urea alone and of urea given with neutral phosphate on the ratio:  $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$*   
*Averages of all periods from experiments on fifteen rabbits*

SUBSTANCE GIVEN	PLASMA CO <sub>2</sub>	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
	vol. per 100 cc.	cc.		mgm. per hour	mgm. per 100 cc.	
Urea.....	51	20	6.3	426	164	2.61
Urea + neutral phosphate..	52	22	6.7	499	161	3.09

TABLE 2

*Comparison of the effect of urea alone and of urea given with neutral phosphate on the ratio:  $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$*   
*Averages of separate periods*

PERIOD	PLASMA CO <sub>2</sub>	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
1. Urea						
	vol. per 100 cc.	cc.		mgm. per hour	mgm. per 100 cc.	
1	48	16	7.0	283	141	2.00
2	53	19	6.2	443	171	2.64
3	52	18	6.0	512	175	2.98
4	52	26	5.8	466	167	2.82
2. Urea + neutral phosphate						
1	48	22	7.2	338	144	2.68
2	52	24	6.6	562	171	3.26
3	55	17	6.8	571	168	3.41
4	54	23	6.3	474	160	2.99

grams of urea per 100 cc. If with such amounts of urea in the blood the ratio is lower when phosphate is given with the urea than when urea is given alone, that fact would be in favor of the hypothesis of a common anatomical mechanism for the excretion of both urea and phosphate, a mechanism which was unable to excrete the urea so



rapidly when it was simultaneously required to deal with the elimination of a large amount of phosphate. If on the other hand the ratio is found to be unchanged when phosphate is given with the urea, such a result might be taken as evidence of the existence of separate mechanisms for urea and phosphate excretion. And finally, an increase in the ratio obtained when phosphate is given with the urea would tend to show in addition that extra-renal factors augmenting excretory activity are more fully mobilized under the double need for elimination.

The same group of 15 rabbits on which the ratio had been measured after 5 grams of urea given with neutral phosphate, was subjected to another series of experiments in which 5 grams of urea alone were administered. The conditions otherwise were the same. The aver-

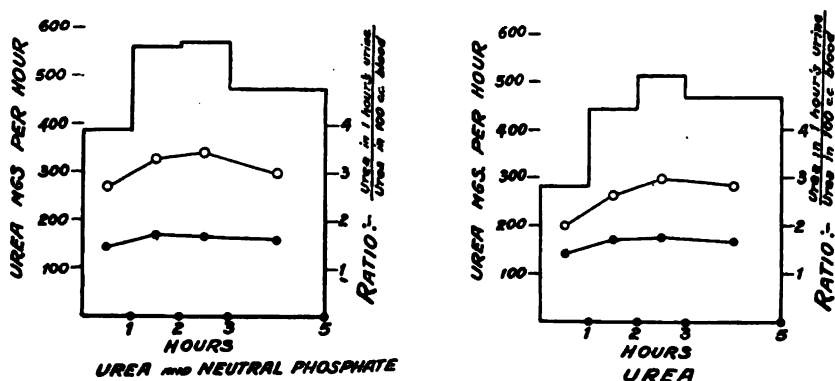


Fig. 1. Showing a slight increase in the urea excreting activity of the kidney when neutral phosphate is given with urea.

ages for the combined and separate periods are shown in tables 1 and 2 and the individual experiments are detailed in table 3 at the end of the paper.

The ratios after urea and phosphate for the four successive periods exceed the ratios after urea alone by  $0.68 \pm 0.15$ ,  $0.61 \pm 0.15$ ,  $0.42 \pm 0.12$  and  $0.11 \pm 0.17$  respectively, i.e., by amounts which cannot well be explained by chance in view of the fact that in all four periods the ratios are greater when both substances were given. The plasma carbon dioxide in both cases was almost the same, so that the effect cannot be accounted for on the basis of a change in the acid base equilibrium. Additional experiments were carried out under the same conditions in order to determine the degree to which phosphate excretion was increased after introducing a balanced mixture of acid and alkaline

phosphate into the stomach. An average hourly excretion of 2.6 mgm. P. was found during the five-hour period when urea alone was given, and 8.0 mgm. when phosphate was given with the urea. The urea excreting activity of the kidney was thus increased while at the same time additional work was being done in the excretion of phosphate.

TABLE 3

*Urea alone*

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period I						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	51.9	18.5	6.3	340	150	2.18
2	39.2	10.0	5.4	290	177	1.64
3	47.5	13.0	6.6	233	123	1.89
4	54.2	20.0	8.0	255	156	1.64
5	49.1	28.5	7.0	495	135	3.67
9	49.3	18.0	7.4	323	204	1.58
10	50.1	17.0	7.4	204	153	1.34
11	45.0	8.0	5.5	132	99	1.34
12	40.2	9.0	7.4	120	78	1.54
13	70.2	25.0	8.7	408	132	3.09
14	48.2	7.5	7.4	228	123	1.86
15	40.3	14.0	7.0	350	150	2.33
16	44.6	21.0	7.5	220	114	1.93
19	40.2	15.0	7.0	360	160	2.14
20	51.5	17.0	7.0	292	153	1.91
Period II						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	51.2	18.5	5.0	350	165	2.12
2	50.4	15.0	6.7	474	189	2.51
3	50.4	13.0	6.0	339	171	1.98
4	56.0	23.0	7.7	500	177	2.82
5	52.3	32.0	4.7	640	150	4.27
9	52.4	19.0	7.0	525	216	2.43
10	49.3	11.5	6.7	233	216	1.08
11	47.2	13.0	4.7	426	150	2.84
12	51.5	27.5	4.7	368	146	2.55
13	71.5	13.0	8.0	450	150	3.00
14	52.1	8.5	6.0	411	150	2.74
15	55.7	16.0	5.6	570	177	3.22
16	56.0	44.0	8.0	414	177	2.34
19	51.0	14.0	5.3	466	171	2.73
20	52.8	18.0	6.3	485	168	2.89

TABLE 3—Continued

RABBIT	PLASMA CO <sub>2</sub>	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
Period III						
	vol. per cent	cc.		mgm. per hour	mgm. per 100 cc.	
1	51.2	20.0	5.0	502	177	2.84
2	47.0	14.0	5.8	519	189	2.75
3	47.3	12.0	6.0	377	177	2.13
4	58.9	19.0	7.0	606	186	3.26
5	54.9	21.5	4.7	621	147	4.22
9	51.3	17.0	7.2	539	219	2.46
10	48.0	15.0	6.3	402	222	1.81
11	47.0	15.5	4.7	510	162	3.15
12	50.3	30.5	4.7	481	153	3.15
13	64.5	11.5	7.6	486	150	3.24
14	52.1	11.0	6.0	521	150	3.47
15	57.2	14.0	6.0	595	180	3.30
16	54.0	30.0	8.0	573	180	3.18
19	45.0	17.0	5.0	452	168	2.69
20	53.5	19.0	6.0	490	159	3.08
Period IV						
1	50.0	41.0	5.0	430	171	2.51
2	53.8	16.0	4.7	440	186	2.36
3	47.5	16.5	6.0	365	165	2.21
4	62.7	20.0	6.7	426	180	2.37
5	53.3	22.0	6.7	527	147	3.59
9	54.9	30.0	6.7	558	192	2.87
10	48.7	36.0	6.0	520	213	2.44
11	50.4	22.0	6.7	495	146	3.44
12	50.1	28.0	6.7	393	162	2.43
13	64.5	19.0	7.0	420	150	2.80
14	51.2	20.0	6.0	560	150	3.73
15	47.8	21.0	6.0	506	153	3.31
16	48.1	40.0	7.7	563	189	3.98
19	49.2	27.0	6.7	345	168	2.05
20	49.3	24.0	6.0	456	141	3.24

## CONCLUSION

The urea excreting activity of the kidney measured during the strain induced by the administration of urea is increased when an additional strain is put on the excretory capacity by the simultaneous administration of a neutral mixture of acid and alkaline phosphate.

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## GASTRIN STUDIES

### III. THE RESPONSE OF THE STOMACH MUCOSA OF VARIOUS ANIMALS TO GASTRIN BODIES

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In the two previous reports (1), (2) published on the distribution of gastrin, we called attention to the characters of the responses of various types of stomachs and determined the distribution of this body (or bodies) in the various tissues.

This method of stimulation of the gastric mucosa gives us an opportunity to investigate some of the more fundamental problems of gastric physiology which can not be attacked through food stimulation. Consequently it is important to extend observations on its secretagogue activity to the more common laboratory animals. If the response proves to be a general property of stomach tissue then generalizations based upon it have an added significance.

#### I. GASTRIN STIMULATION

*Cats.* In a former paper (1) two observations on cats with Pawlow stomachs showed no increase in the rate of secretion of gastric juice after gastrin injection, but a distinct increase in the pepsin concentration. In the table below (table 1) it is definitely shown that cats respond not only as to increase in concentration of acid but also as to volume of gastric juice.

It will be noted that cat I failed to respond until the cord had been sectioned; cat II also presented two negative experiments. Since these studies were undertaken earlier in the work using less active preparations, they are to be considered rather as evidence that the stomach of the normal cat is much more refractory than that of the dog. The insusceptibility of the cat's stomach comes into evidence

again, when they are dressed, and one collects the resulting spontaneous secretion. For it is the rule that the juice obtained consists of viscid neutral mucus, varying in quantity from 1 cc. per hour to 1 cc. collected in 4 to 5 hours. The absence of acid in this spontaneous secretion is in striking contrast to the conditions found in dogs; for in a dog with a gastric fistula one may at times by simply dressing observe the presence of a secretion of 20 to 30 cc. of juice per hour containing

TABLE 1  
*Gastric fistulae*

	CAT I. CORD CUT AT 2ND THORACIC			CAT III. DOUBLY VAGOTOMIZED			CAT IV. NORMAL			CAT V. NORMAL		
	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid
			Free Total			Free Total			Free Total			Free Total
	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent
Hour preceding.....	2.5	0.31	0.35	1.6	0.02	0.10	1.9	0.00	0.03	1.0	0.10	0.17
Dose.....	1 cc. of gastrin (SI)			1 cc. of gastrin (Sh,p. 40)			1 cc. of gastrin (Sh,p. 40)			1 cc. of gastrin (Sh,p. 40)		
First hour.....	10.0	0.47	0.51	9.8	0.39	0.43	9.5	0.28	0.32	9.8	0.37	0.43
Second hour.....	10.0	0.53	0.54	3.5	0.33	0.41	2.2	0.30	0.37	7.8	0.47	0.53
Third hour.....	4.6	0.50	0.53	1.0	0.20	0.27	1.8	0.18	0.26	3.0	0.44	0.47

Additional data:

Cat I. Failed to respond to 1 injection of gastrin preceding the section of cord. Three other experiments after cord was sectioned confirm the one cited above.

Cat II. Two negative experiments.

Cat III. Two other confirmatory experiments.

Cats IV and V. One other confirmatory experiment on each. The gastrin solutions used here have been freed to a great extent from proteins by alcohol and basic lead acetate precipitations.

0.5 per cent hydrochloric acid, and it may require 2 to 3 hours for this secretion to subside.

This refractory condition following injections may be simply due to vascular changes, as is suggested later in the discussion.

Attention is called to cat III which was doubly vagotomized and which confirms the activity of this type of stomach in dogs as previously reported.

*Rabbits.* Most observers are familiar with the great difficulty experienced in freeing the stomach of these animals of food. It was our practice to sew the stomach of the animal to the abdominal wall over a fairly wide area and then on the following day to make an opening into the lumen by means of a cautery. If the opening is sufficiently large the stomach can be eventually emptied by the use of a fine stream of water. These animals responded readily in our experiments, two of which are cited.

*Rabbit I. July 10*

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
3:30	Stomach washed out		
4:10	3.0	0.00	0.02
4:10	Injected 1 cc. gastrin	"Sh filtrate, p. 40" intramuscularly	
5:10	26.4	0.17	0.20
6:10	25.0	0.36	0.41

*Rabbit II. July 12*

3:00	Stomach washed out		
4:25	Collection begun		
4:55	7.5	0.00	0.04
5:25	7.7	0.00	0.03
5:37	Injected 1 cc. gastrin	"Sh filtrate, p. 40" intramuscularly	
6:37	33.0	0.20	0.27
7:37	25.0	0.12	0.19

*Guinea pigs.* Much difficulty was experienced in obtaining even qualitatively satisfactory experiments on the guinea pig. The emptying of the stomach is attended with a good deal of shock, so that the animals remain rather quiet and depressed after the manipulation. Furthermore, they also more readily show toxic manifestations from the injections. This may possibly be explained as due to improper gradation of the dose.

The following experiment is offered as one of qualitative value in establishing the activity of the preparation.

In view of the difficulties mentioned above it was not considered necessary for our present purposes to seek for more conclusive results.

In the control hour preceding the injection the quantity of juice was 2.5 cc. with an acidity too small to titrate; in the hour following 4.5 cc.

were secreted with a total acid reaching 0.11 per cent. A second injection of double the amount was then given with no increase in quantity, but a further rise in total acid to 0.16 per cent.

*Ducks.* Ducks were selected for the avian stomach because of their large size and the relative ease with which they can be kept in captivity. A fistula was made into the crop and a rubber tube inserted which tube could be removed at the time of the experiment. Food was removed from the cages the night preceding the experiment. At stated intervals (half-hour or hour periods) the proventriculus was aspirated with a soft perforated gum rubber tube attached to a syringe. The protocols below show definite time intervals but these are to be considered approximations, for often 10 to 12 minutes were consumed in manipulations attendant upon the aspirations. One duck (no. II) died the night following the injection of a rather heavy dose, the others remained in excellent condition. The breast muscles offer a convenient site for the injection.

*Duck I*

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
9:15	Stomach aspirated; nothing returned		
9:15	2 cc. gastrin "SO" intramuscularly		
10:15	8.5	0.12	0.20
11:15	10.2	0.22	0.26
12:15	4.9	0.20	0.24

*Duck II*

8:30	Stomach washed out		
9:30	1.0	0.06	0.12
9:30	3 cc. gastrin "SO" intramuscularly		
10:30	27.0	0.20	0.26
11:30	26.0	0.24	0.28
12:30	12.5	0.25	0.31

*Duck III*

8:35	Stomach washed out		
9:35	0.5	0.00	0.09
9:35	2 cc. gastrin "SO" intramuscularly		
10:05	0.7	0.37	0.45
10:05	1 cc. gastrin "SO" intramuscularly		
10:35	15.6	0.32	0.37
11:35	28.0	0.21	0.26
12:35	25.0	0.18	0.23

These active responses as shown in the protocols present all the characteristics as to quantity, acid and duration, of the mammalian secretion.

*Turtles.* With these animals our results have been entirely negative. First intravenous injections into the abdominal veins of pithed preparations were made and the stomach tested at varying intervals with congo red paper. Next several animals were injected intramuscularly with a known active preparation, and after varying intervals pithed. The stomach contents were then tested with congo red paper. In no case was an acid reaction developed. We then in routine autopsied all turtles used in the course of a rather extended piece of pharmacological research in progress in the same building and tested their stomachs for acid. These animals were fed liver daily and their stomachs always contained pieces thereof, but we were never able to demonstrate an acid reaction in the liver debris or in the mucus on the stomach wall. In the light of these findings we feel that our negative results are to be attributed to the resting state of the stomach in captivity and to the hot weather during which we performed the experiments.

*Frogs.* Gastric fistulae were made in large bull frogs, measuring approximately 30 to 36 cm. in length. These keep well in captivity, the wound healing readily. Indeed if they are not used regularly the fistula will close entirely. Injections were made into the dorsal lymph sac.

October 23, 1916

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
12:25	0.3 glairy mucus, acid to congo red, aspirated with a perforated rubber on a syringe		
12:25	1.25 minims gastrin "SO" in dorsal lymph sac		
1:25	1.9	0.26	0.32
2:25	1.7	0.29	0.35
3:25	0.4	0.16	0.25

October 24, 1916

10:30	Nothing returned by aspiration. Stomach then wiped out with a pledget of congo red paper, no reaction		
10:35	1.66 minims gastrin "SO" in dorsal lymph sac		
11:35	2.4	0.22	0.29
12:35	1.4	0.27	0.30
3:30	1.3	0.23	0.30



The above experiments present conclusive evidence of the secretory response of the frog's stomach.

A number of smaller frogs such as are customarily used in laboratory work were injected, afterwards pithed and stomach tested with congo red paper, but in no case was a positive response noted.

*Goat.*<sup>1</sup> A female goat was operated on November 16, 1915, with the establishment of an Heidenhain stomach. A total of five experiments was performed on the animal in the course of the next month.

TABLE 2  
*Histamine-di-hydrochloride injected intramuscularly*

	PAWLOW ACCESSORY STOMACHS											
	Dog I			Dog II*			Dog X			Dog VIII		
	Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid	
		Free	Total		Free	Total		Free	Total		Free	Total
cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent	
Hour preceding.....	0.6	0.04	0.07	0.8	0.05	0.12	6.0	0.29	0.31	2.0		0.3
Injection.....	4 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride		
First hour.....	3.8	0.26	0.31	4.5	0.26	0.36	8.5	0.47	0.50	9.0	0.38	0.48
Second hour.....	4.0	0.50	0.55	3.5	0.39	0.45	2.5	0.12	0.13			
Third hour.....	0.5	0.40	0.46	0.2								

\* Heidenhain stomach.

The response was in no case striking. However, there was constantly a slight increase in the acid and an inconstant increase in pepsin and quantity output following the injection. The evidence seems sufficient to warrant the statement that some stimulation really occurred.

<sup>1</sup> The authors are deeply indebted to A. F. Schalk of the Veterinary School of the North Dakota Agriculture College for these experiments and the preparation of the test animal.

## II. HISTAMINE STIMULATION

The character of the response under histamine resembles closely that given by the gastrin preparations as shown by tables below. While as yet no attempt has been made to make a comparative study of the phenomena associated with the injections of the two bodies, since such a study would be more valuable when a pure gastrin product is obtained; still it has often seemed that the subjective symptoms associated with histamine administration were more pronounced. The

TABLE 3

*Histamine-di-hydrochloride injected intramuscularly*

	DOG XI. GASTRIC FISTULA			DOG IV. GASTRIC FISTULA VAGI CRUSHED ONE YEAR PRE- VIOUSLY			DOG V. GASTRIC FISTULA VAGI CRUSHED ONE YEAR PRE- VIOUSLY			DOG VI. VAGOTO- MIZED GASTRIC FISTULA			DOG VII. VAGO- TOMIZED GASTRIC FISTULA		
	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid	Juice		Hydro- chloric acid
	Free	Total		Free	Total		Free	Total		Free	Total		Free	Total	
Hour pre- ceding..	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent
	12.6	Neg.	+	8.5	0.36	0.42	9.5	0.37	0.43	7.0	0.11	0.25	3.6	0.11	0.18
Dose.....	1 mgm. his- tamine (HCl) <sub>2</sub>			1 mgm. his- tamine (HCl) <sub>2</sub>			1 mgm. his- tamine (HCl) <sub>2</sub>			0.5 mgm. histamine (HCl) <sub>2</sub>			0.5 mgm. histamine (HCl) <sub>2</sub>		
First hour...	54.5	0.50	0.54	62.5	0.43	0.47	25.5	0.42	0.47	16.8	0.25	0.34	18.5	0.26	0.33
Second hour...	14.0	0.45	0.48	35.0	0.45	0.50	2.0			5.6	0.26	0.39	1.7	0.23	0.31

animals were invariably restless and showed noticeable evidences of discomfort for 15 to 30 minutes following the injection. Superficial vasodilatation as shown by the reddened nose, conjunctiva and mucosa of the mouth always was definite and frequently marked.

The limits of the dosage have not been determined accurately, but it is safe to say that 0.5 mgm. histamine-di-hydrochloride will produce a satisfactory response as shown by dogs VI and VII. These two animals had been doubly vagotomized, a procedure which our experience has taught us always renders the dog's stomach more refractory.

*Cats.* Two positive experiments are recorded below.

*Cat III (July 19, 1916) doubly vagotomized*

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
1:25	Dressed		
2:25	1.0	0.19	0.35
3:05	1 mgm. histamine-di-hydrochloride intramuscularly		
4:05	13.0	0.22	0.29
5:05	6.2	0.45	0.48

*Cat V. Normal (August 7, 1916)*

3:00	Dressed		
4:00	2.2		0.03
4:00	1 mgm. histamine-di-hydrochloride intramuscularly		
5:00	12.0	0.21	0.26
6:00	9.0	0.39	0.45

*Rabbits.* Rabbits appear to present a slight peculiarity in the time element of the secretion. By references to table 4 it will be noted that the maximum quantity as well as the high point in the acid comes in the second hour. We have no suggestion based on our observations to offer in explanation. However the question is discussed more completely later. Negative results were obtained in case of one animal (no. II) which was in such poor condition that he died a few hours after the injection. So we feel that this particular case is without significance.

*Guinea pigs.* The experimental difficulties in handling these animals have already been discussed. Two experiments of qualitative value are presented.

In the first case a control period of 3 hours showed a rate of 0.2 cc. with no titratable acid, the hour following an injection of 1 mgm. histamine hydrochloride gave 0.4 cc. juice with 0.11 per cent free and 0.15 per cent total hydrochloric acid.

In a second experiment the control period showed no acid while the hour following the injection gave 0.07 per cent free and 0.11 per cent total hydrochloric acid. There was no increase in quantity.

TABLE 4

*Histamine-di-hydrochloride injected intramuscularly*

	RABBIT III			RABBIT III			RABBIT IV		
	Quan- tity	Hydro- chloric acid		Quan- tity	Hydro- chloric acid		Quan- tity	Hydro- chloric acid	
		Free	Total		Free	Total		Free	Total
	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent
Hour preceding.....	0.2			4.0	0.14	0.21	0.8		
Injection.....	1 mgm. his- tamine-di- hydro- chloride			1 mgm. his- tamine-di- hydro- chloride			1 mgm. his- tamine-di- hydro- chloride		
First hour.....	1.8	0.15	0.33	4.6	0.30	0.36	7.8	0.25	0.37
Second hour.....	11.0	0.36	0.48	30.0	0.39	0.45	14.5	0.43	0.51
Third hour.....	12.0	0.32	0.47						

*Ducks.* The stimulation of the avian stomach is shown in the following protocols.

*Duck I. October 1, 1916*

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
7:45	Stomach aspirated, nothing returned		
8:00	0.5 mgm. histamine-di-hydrochloride		
8:30	4.2	0.42	0.29
8:30	0.5 mgm. histamine-di-hydrochloride		
9:30	22.0	0.24	0.29
10:30	4.5	0.16	0.23

*Duck III. October 1, 1916*

8:05	Stomach aspirated, nothing returned		
8:10	1 mgm. histamine-di-hydrochloride		
9:10	10.0	0.21	0.26
10:10	3.8	0.06	0.14

*Frogs.* The experiments were conducted as previously described and with the following results.

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
October 25, 1916			
9:40	Stomach aspirated, nothing returned. Wiped out the stomach with a pledget of congo red paper and no acid reaction shown		
9:50	$\frac{1}{2}$ mgm. histamine-di-hydrochloride		
10:50	0.5	0.20	0.26
12:00	0.9	0.23	0.29
12:00	$\frac{1}{2}$ mgm. histamine-di-hydrochloride		
1:00	0.9	0.27	0.30
2:00	1.0	0.23	0.29
October 26, 1916			
9:30	Stomach aspirated, nothing returned. Wiped out the stomach with a pledget of congo red paper, no acid reaction		
9:30	$\frac{1}{2}$ mgm. histamine-di-hydrochloride		
10:30	1.0	0.20	0.24
11:30	1.4	0.27	0.30
1:45	0.3		

### III. TYRAMINE HYDROCHLORIDE

Although the experiments using this compound are incomplete and inconclusive, yet it seemed best to report our findings to date. It is our intention to complete them as soon as a sufficient quantity of the compound can be synthesized.

Protocols of two positive experiments follow:

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
<i>Cat III. August 18, 1916. Doubly vagotomized</i>			
	cc.	per cent	per cent
1:10	Dressed		
2:10	0.7	0.04	0.13
2:25	5 mgm. tyramine hydrochloride (Hoffmann-La Roche)		
3:25	6.5	0.19	0.27
4:25	1.2	0.17	0.24
5:55	2.6	0.10	0.18
<i>Cat IV. August 9, 1916. Normal</i>			
10:30	Dressed		
11:30	0.4	0.00	0.01
12:00	5 mgm. tyramine hydrochloride (Hoffmann-La Roche)		
1:00	5.0	0.13	0.16
2:00	2.5		0.06
3:00	0.5		

Other experiments with tyramine hydrochloride on cats may be summarized thus:

*Cat 3.* Dose  $7\frac{1}{2}$  mgm. positive;  $2\frac{1}{2}$  mgm. negative.

*Cat 4.* Dose  $7\frac{1}{2}$  mgm. positive;  $2\frac{1}{2}$  mgm. negative.

*Cat 5.* Dose 5 mgm. negative.

*Cat 6.* Dose  $7\frac{1}{2}$  mgm. positive; 5 mgm. questionable;  $2\frac{1}{2}$  mgm. positive.

#### DISCUSSION

In our two former papers (1), (2) on the distribution of gastrin activity, we reported that it occurs in approximately the same concentrations in stomachs and duodenal mucosa and in the thyroids, providing we do not try to remove the depressor activity by absolute alcohol extraction. Using the same methods we concluded that at times, and for as yet unknown reasons, we find even higher concentrations of the gastrin activity in the liver and pancreas.

We here find the gastric secretory mechanisms of various animals to be stimulated to more or less extent by both histamine and gastrin solutions when injected intramuscularly. In the turtle we were not able to bring about secretion by either substance or by food.

The guinea pig reacted very irregularly and poorly. This may be due to the extreme susceptibility of this animal to histamine as shown by other observers. Thus, Dale and Laidlaw (3) call attention to the very rapid death after the intravenous injection, and conclude that death is due to constriction of the bronchioles together with increased pulmonary pressure. This pulmonary involvement could however be avoided and an actual general rise in blood pressure due to general vasoconstriction could be observed if previous to the injection of histamine the animal were kept under prolonged anesthesia. Other observers have noted diametrically opposite physiological results on various animals as regards blood pressure and also distinctly variable results as to contraction and relaxation of smooth muscle preparations from various organs when treated with histamine. Thus Barbour (4) considers histamine to cause a fall in blood pressure particularly in carnivora, and that positive or negative results on isolated smooth muscle preparations by epinephrin, tyramine and histamine are determined by the quantitative character of the vasoconstrictor and vasodilator fibers to the particular organ in question. It is well known that the virgin or non-pregnant uterus of the rabbit, dog, ferret, monkey or man always contracts after adrenalin treatment

whereas in the cat, guinea pig, and rat this may cause a relaxation and that only the pregnant uterus is caused to contract. The same theory as to the predominance of one or the other form of nerve endings has frequently been resorted to in attempts to explain these results. Cow (5), however, postulates that in the pregnant animal the pituitrin concentration in the blood is higher and hence the pregnant uterus reacts more readily to injected adrenalin or pituitrin. In other words a previous "sensitization" of the tissues with pituitrin (histamine?) (13) renders smooth muscle more susceptible to uniform action by pilocarpine, ergot, ergotoxin, pituitrin, tyramine and adrenalin. Cow has actually shown that by previous feeding of pituitary substance or by previous treatment with pituitrin solutions the non-pregnant uterus of the cat can be made to react like a pregnant uterus when later treated with the drugs named. Just how this "sensitization" is brought about, or whatever the action may be, has not been explained as far as we know. Fröhlich and Pick (6) made observations along somewhat similar lines, but with entirely different and less consistent results. Handowsky and Pick (7) conclude from their studies with the Trendelenburg frog muscle method that histamine (1:1000), tyramine (1:1000) and Witte's peptone (1:100) all act as vasodilators on this preparation, providing the muscle is first put into the vasoconstrictor condition. While these observations do not bear directly on the point in question here, still they are suggestive and some such factors as referred to may explain our peculiar results with guinea pigs and rabbits.

In rabbits we observed less toxic action by gastrin than in guinea pigs, but the time of maximum gastric secretion after histamine is delayed until the second hour, whereas in most of the animals studied we find the maximum rate of secretion in the second thirty minutes after the injection. Possibly the delay may be attributed to the earlier vascular changes leading to an anemia around the glands and that the otherwise favorable secretory action could not manifest itself until later. With gastrin, however, the rabbits show their maximum secretion in the first hour or at any rate a response of the same order as that of the second hour. These observations suggest that gastrin solutions do not contain histamine or, if they do, that the physiological action thereof is modified by some other constituent. If our interpretation of the delay is correct we may restate the proposition by saying that the gastrin solutions do not contain a substance which produces vascular changes unfavorable to secretion. Possibly the more refractory state of the cat's stomach is attributable to these same vascular factors, that is, initial vasoconstriction.

The observations by Dale and Richards (8) indicate a remarkable vascular action by histamine. Apparently it acts upon two distinctly different anatomical structures in the vascular system. It causes a transient vasoconstriction in the arterioles and a more delayed and prolonged vasodilating action on the capillaries due to inhibition of their tone. The main action then consists in opening up new capillary areas together with permeation of the capillary wall by blood plasma. This latter property enables us to understand how the optimum vascular conditions for secretion may be produced by histamine. Dale and Richards' observations indicating a loss of plasma and an increase in concentration of red corpuscles in the blood, as a result of histamine, have also been observed by Simon (9), who noted an eosinophilia after injection of pancreatic secretin solution. Downs and Eddy (10) also recently confirmed the findings of Dale and Richards in that they noted a distinct increase in the concentration of the formed elements in the blood after partaking of food and also after the injection of pancreatic secretin. However, Simon, as well as Downs and Eddy, consider their results as due to an actual increased production of blood cells.

We do not claim that histamine has a specific stimulating effect on gastric secretion for it is well established by Dale and Laidlaw (11) that the salivary glands as well as the pancreas are stimulated to secretion by histamine. There are other studies which indicate a very close similarity if not an identity between histamine, pancreatic secretin and gastrin. Thus, Barger and Dale (12) using a 0.1 per cent HCl extraction, report the isolation of "crystals quite similar to histamine dipicrate" from the small intestines of the ox, under conditions which precluded post-mortem bacterial changes in the tissue. Various observers have considered this as showing that histamine is identical with secretin, although the proof is by no means conclusive either for or against this view. Similarly one might argue that gastrin and histamine are identical and particularly so in view of the recent observations of Abel and Kubota (13), who isolated histamine picrate from gastric mucous membrane.

In regard to the question of the identity of pancreatic secretin and histamine we have the findings of Dale and Laidlaw (11). These authors report one experiment in which it is shown that if doses of histamine and pancreatic secretin which depress the blood pressure to the same level be injected, then the flow of pancreatic juice resulting from the injection of secretin is much greater than that resulting from histamine stimulation. They were, however, not able to prepare a secre-



tin solution free from depressor action. We have not studied the relation of blood pressure to gastric secretion as a result of gastrin and histamine since all of our experiments were made without the use of anesthetics. We have, however, the general impression that histamine is more toxic and causes more marked peripheral vascular changes when we compare equivalent doses from the standpoint of secretagogue action. These general observations together with others noted above and to be reported in later papers, lead us to believe that histamine and gastrin probably are not identical.

We have no conclusive data to offer as to the action of tyramine. Its more definite vasoconstrictor action probably outweighs any secretory stimulating power that it possesses. Our two positive experiments merely suggest that if the vascular conditions were otherwise right for secretion, the response probably would always be positive.

#### CONCLUSIONS

1. Gastrin and histamine cause gastric secretion in the dog, cat, rabbit, guinea pig, duck and frog.
2. Gastrin causes secretion in goat; histamine has not been tested on this animal.
3. The turtle's stomach did not contain at any time free acid (as tested by congo red) following injection of gastrin intravenously and intramuscularly or the ingestion of food.
4. The results in the guinea pigs are not striking or clear cut, due to their general susceptibility to the injected substances.
5. In the rabbit the maximum secretion from gastrin occurs in the first hour, from histamine in the second. This may be attributed to the preliminary vasoconstriction caused by histamine.
6. The pharmacologic action of histamine on the capillaries produces ideal conditions for secretion.
7. Histamine appears to have the property of exciting glandular tissue in general (pancreas, salivary gland and stomach).
8. The behavior of gastrin does not conform sufficiently to histamine to warrant the conclusion that they are the same substance.
9. Tyramine caused secretion in cats inconstantly, but failed in all other animals.

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## GASTRIN STUDIES

### IV. THE RESPONSE OF THE STOMACH MUCOSA TO FOOD AND GASTRIN BODIES AS INFLUENCED BY ATROPINE

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The stimulating effect of morphine and pilocarpine (1) on gastric activity has been known for a number of years. The authors (2) have more recently extended Edkins' (3) observations on gastrin and have added to the list another substance, histamine, which when injected intramuscularly, causes gastric secretion. A large list of other substances has been reported by investigators at different times as capable of causing secretion. However, these have usually been administered orally and the evidence in favor of their activity is neither clear nor convincing. It seemed advisable to study the effect of atropine on the secretions caused by food and by gastrin bodies under which term for the present histamine is included. It was thought that such studies might enable us to estimate more accurately the value of these substances as stimulating agents and at the same time throw some light on the more fundamental problems of gastric secretion.

*Effect of atropine on food stimulation.* Riegel (4) has reported that atropine causes a decrease in the quantity of juice and in the height of acid after a standard meal of one liter of milk. He further showed that it decreased the secretion in cases clinically diagnosed as ulcers. From this and other studies into gastric motility as influenced by atropine and belladonna these bodies have assumed a definite value in the diagnosis of gastric ulcer and its management. We have substituted for Riegel's milk meal one which we hoped would give a maximum secretory effect, and with this have extended his observations. The meal consisted of 250 grams of lean meat, hashed through the meat chopper three times, 300 cc. of water and 5 grams of sodium chloride. The mixture was boiled long enough to change its appear-

ance to a uniform brown. Its stimulating power is shown in the protocols below.

*Dog VIII. Pawlow stomach*

*Experiment 1.* Fed standard diet. Hourly average for the first five hours was 12.7 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	per cent	per cent
Fifth hour.....	10.5	0.33	0.41
Sixth hour.....	3.0	0.24	0.38

*Experiment 2.* Fed standard diet. Hourly average for the first five hours was 16.0 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	per cent	per cent
Fifth hour.....	16.0	0.42	0.51
Sixth hour.....	6.0	0.29	0.40

*Dog IX. Pawlow stomach with splanchnic nerves sectioned.*

*Experiment 1.* Fed standard diet. Hourly average for the first five hours was 12.3 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	per cent	per cent
Fifth hour.....	13	0.44	0.50
Sixth hour.....	18	0.47	0.51

*Experiment 2.* Fed standard diet. Hourly average for the first five hours was 26.0 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL ACID
	cc.	per cent	per cent
Fifth hour.....	20	0.32	0.45
Sixth hour.....	11	0.30	0.37

We may conclude that normally the stomachs of these experimental animals displayed good secretory activity for at least six hours following the test meal used.

*Effect of toxic doses of atropine on the secretion.* In the first experiments a rather large dose of atropine was given which proved to be a

generally toxic one. It was only after twenty-four hours that the animals could be interested in food, while the effect on the pupil lasted for several days. The prostration with this dosage was profound.

Reference to table 1 shows that the effect on the quantity secretion is immediate, and the effect on the percentage of acidity occurs after thirty minutes. The dose was then reduced in order to see whether the inhibitory dose must be a generally toxic one. It was found that 1 mgm. of atropine sulphate (0.12 mgm. per kgm. body weight) would inhibit the secretion of the gastric juice over a period of five hours following the injection. No attempt was made to follow the secretion

TABLE 1

*Effect of toxic dose of atropine on secretion from food stimulation*

TIME INTERVALS	DOG VIII			DOG IX		
	Quantity	Hydrochloric acid		Quantity	Hydrochloric acid	
		Free	Total		Free	Total
Fed standard meal						
	cc.	per cent	per cent	cc.	per cent	per cent
First hour.....	9.0	0.08	0.35	8.5	0.22	0.42
Second hour.....	18.0	0.34	0.49	16.0	0.36	0.49
Third hour.....	18.0	0.38	0.46	19.8	0.30	0.46
Injected 2 mgm. atropine sulphate per kilo body weight, intramuscularly						
Next half-hour.....	2.4	0.34	0.43	2.2	0.37	0.47
Next hour.....	0.7	0.00	0.10	0.4	0.00	0.29
Next hour. ....	0.4	0.00	0.06	0.2	0.00	
Next hour.....	0.5	0.00	0.09	0.3	0.00	

longer and determine the exact time at which it would return. Such a period we took as evidence of definite and complete inhibition. This dose did not produce any other obvious symptoms. After the injections the animals lay down as usual, responded to petting and appeared normal in all respects, except for an occasional stretching of the mouth and licking of the nose. Hence we may say that an inhibitory dose need not be a generally toxic one.

*Qualitative changes in the juice after atropine injections.* By using still smaller doses one can analyze more clearly the steps in the inhibition. Tables 2 and 3 give the results of ten experiments carried out for this purpose. The first effect appears to be one on the quantity

secretion. Reference to tables 2 and 3 in which 0.05 and 0.025 mgm. doses were used shows that in all cases there is a sudden drop in the

TABLE 2

*Effect of atropine on quantity partition of gastric juice after standard meal.  
Atropine sulphate injected intramuscularly*

TIME INTERVALS	DOG VIII			DOG IX			DOG VIII		DOG IX		
	Quantities of juice			Quantities of juice			Quantities of juice		Quantities of juice		
One hour.....	6.0	7.0	3.0	13.0	9.5	13.5	4.0	5.0	2.0	3.5	
One half-hour.....	10.0	7.0	3.0	12.0	9.0	10.0	4.6	2.7	2.5	8.4	
	0.05 mgm. atropine sulphate			0.05 mgm. atropine sulphate			0.025 mgm. atropine sulphate				
One half-hour.....	3.3	1.8	1.7	3.0	2.0	3.4	2.5	2.0	1.0	3.8	
One hour.....	4.0	5.2	2.2	6.8	9.0	11.0	2.9	2.2	1.5	8.3	
One hour.....	6.0	7.8	2.8	19.0	17.1	17.4	2.5	3.8	3.9	11.5	
One hour.....		6.9	2.6		18.7	14.4	2.5	4.3	9.5	11.0	

TABLE 3

*Effect of atropine on total acid partition in the gastric juice after standard meal*

TIME INTERVALS	DOG VIII			DOG IX			DOG VIII		DOG IX		
	Per cent total hydrochloric acid*			Per cent total hydrochloric acid			Per cent total hydrochloric acid		Per cent total hydrochloric acid		
One hour.....	0.48	0.45	0.36	0.55	0.51	0.55	0.34	0.36	0.20	0.55	
One half-hour.....	0.48	0.51	0.48	0.58	0.57	0.58	0.46	0.48	0.40	0.58	
	0.05 mgm. atropine sulphate			0.05 mgm. atropine sulphate			0.025 mgm. atropine sulphate				
One half-hour.....	0.47	0.51	0.46	0.54	0.58	0.58	0.45	0.43	0.33	0.55	
One hour.....	0.36	0.41	0.27	0.46	0.46	0.51	0.37	0.36	0.36	0.51	
One hour.....	0.43	0.45	0.35	0.51	0.55	0.64	0.31	0.42	0.50	0.56	
One hour.....		0.46	0.33		0.57	0.58	0.27		0.50	0.55	
One hour.....		0.42			0.58	0.58					

\* Since the free acid curve figures followed so closely those of total acid they are omitted for the sake of brevity.

quantity output. This comes on in the first half-hour and with these doses persists for one and one-half to two hours. The acidity (table 3)

is only slightly reduced. Indeed one may consider the acidity changes well within the limits of variation of normal stomachs on a test meal. However if the dose of atropine be increased to 0.1 to 0.2 mgm. then the drop in acid is unmistakable.

These facts are quite clearly illustrated in the attached protocol.

*Dog VIII. Pawlow stomach*

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	per cent	per cent
10:20	Fed the standard meal		
11:20	15.5	0.46	0.49
12:20	17.0	0.53	0.55
12:24	Injected 0.2 mgm. atropine sulphate intramuscularly		
12:54	1.6	0.48	0.55
1:24	0.4	0.27	0.38
2:24	1.2	0.04	0.07
3:24	1.4	0.00	0.02
4:24	1.0	0.12	0.21

It is clear that a 50 to 80 per cent reduction in the quantity output may be made without affecting the acid concentration. When, however, the reduction is greater than this, then changes in the acid are noted.

*Pepsin.* If we measure the pepsin content by Mett's tubes and express the concentration in the square of the millimeter of albumin digested by the juice diluted to twice the original volume in 0.3 per cent HCl, then the following table (table 4) shows the immediate and definite effect of the intramuscular injection of such small doses as 0.05 to 0.025 mgm. atropine sulphate.

In a normally secreting stomach as the quantity of juice is increased the concentration of peptic units falls. Thus in dog VIII, the quantity rose from 4 cc. per hour to 9.2 cc. per hour, while the pepsin concentration expressed in squares of millimeters of albumin digested in Mett's tubes fell from 12.25 to 2.56. In the case of dog IX, the quantity rose from 3.5 cc. to 16.9 cc. while the concentration fell from 5.76 to 2.72. At the close of a period of normal digestion, the reverse of this occurs; the quantity drops and the concentration per cubic centimeter rises. This condition is in sharp contrast to that occurring after the injection of atropine. Here both the quantity of gastric juice and the pepsin concentration show an immediate reduction. In other words, we have a definite inhibition or toxic action on the secretory mechanism. We

may then place the secretion of pepsin along with that of the quantity, and say that when these two are affected the mechanism has received a first degree or first level damage. When the acid is lowered, the damage is of the second degree or to a second lower level.

*Effect of atropine on gastrin stimulation.*<sup>1</sup> In studying this phase of the problem an attempt was made to secure a dose of gastrin which would throw the gastric secretory mechanism into a uniform rate of activity, and then give increasing doses of atropine.

The stimulating dose of gastrin chosen was 0.25 cc. of Sb6B. This was a partly purified preparation obtained by removing proteins and other inert substances by alcohol and basic lead acetate precipitations.

TABLE 4

*Effect of atropine on pepsin content under food stimulation*

BEFORE	DOG VIII			DOG IX		
One hour.....	12.25	4.41	4.42	6.76	5.76	10.89
Second hour.....	2.56	1.21			2.72	
Atropine dosage.....	0.025 mgm.		0.05 mgm.	0.025 mgm.		0.05 mgm.
One to two hours.....	1.64	0.12	1.0	4.84	3.06	5.76
Three to four hours.....	1.21	0.06	1.21	2.96	1.69	14.44
	8.51	1.44		4.41	1.69	16.81
				6.25	1.69	

Note the return of peptic content to normal as the effect of the atropine wears off.

The gastrin was given three-fourths to one hour after the atropine. Both the atropine and the gastrin were injected intramuscularly. The results are shown in table 5.

The animal began to show signs of toxicity with 0.8 mgm. atropine but took food at the end of the experiment. It will be noted that the secretion was inhibited quite as much by the 0.2 as 0.8 mgm. and that it took a generally toxic dose, 2.4 mgm., practically to abolish the secretion.

<sup>1</sup> Since completing this work our attention was called to an article by Maydell (Dissert. Kiev, 1917, 170) in which according to the reviewer "atropine diminishes the secretory effect of gastric secretin" (cited from Physiological Abstracts, 1917-18, II, 146. Abstr. no. 1700).



TABLE 5

*Effect of increasing doses of atropine on gastrin stimulation*

ATROPINE SULPHATE	GASTRIN	TIME	QUANTITY OF JUICE	HYDROCHLORIC ACID	
				Free.	Total
<i>mgm.</i>	<i>cc.</i>		<i>cc.</i>	<i>per cent</i>	<i>per cent</i>
None	0.25	1st hour	3.0	0.23	0.26
		2nd hour	0.25	0.22	0.26
0.2	0.25	1st hour	1.8	0.25	0.27
		2nd hour	0.5	0.00	0.09
0.8	0.25	1st hour	1.7	0.26	0.30
2.4	0.25	1st hour	0.6	0.18	0.22
		2nd hour	0.2		

It was next of interest to know the effect on the secretion of increasing the dose of gastrin. The protocols of two experiments follow.

*Dog III. Pawlow stomach*

TIME	JUICE	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
Control experiment			
	<i>cc.</i>	<i>per cent</i>	<i>per cent</i>
8:50	Dressed		
9:50	0.3	0.00	trace
9:50	1 cc. gastrin (Sb6B) intramuscularly (4 times the minimal secretory dose)		
10:50	6.2	0.28	0.37
11:50	8.8	0.46	0.48
12:35	4.1	0.43	0.46
Atropine experiment on the same dog			
10:10	2.4 mgm. atropine sulphate intramuscularly		
11:10	1 cc. gastrin (Sb6B) intramuscularly		
12:10	3.5	0.23	0.27
1:10	5.3	0.41	0.47
2:10	2.4	0.38	0.41

The dose of atropine, 2.4 mgm., which had completely inhibited the secretion from 0.25 cc. of gastrin has depressed the secretion only (40 per cent) when the dose of gastrin was quadrupled. Duplicate

experiments were run on dogs IV and V with striking confirmation of the findings expressed above.

Working in the ranges of toxic doses the following experiments are of interest as showing the quantitative antagonistic relations of the two preparations.

*Effect of atropine on histamine stimulation.* The quantitative antagonism of the gastrin series also holds for histamine and atropine. This is beautifully illustrated in table 7 below.

The question naturally arises whether it is possible, by giving doses of atropine sufficiently toxic, to render the gastric mechanism insensitive to histamine stimulation. For this two gastric fistulae animals were selected since a qualitative rather than an absolute quantitative result was desired. The protocols of experiments in which 20 to 21 mgm. of atropine sulphate (approximately 3 mgm. per kilo body weight) were administered are appended. In these an unquestionable secretion is present, although it is reduced in quantity.

*Dog VII. Gastric fistula*

TIME	JUICE	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	per cent	per cent
9:45	Dressed		
10:15	2.0	0.03	0.01
11:15	3.0	0.00	0.01
11:30	Injected 20 mgm. atropine sulphate (intramuscularly)		
12:00	2.0	0.00	0.03
12:00	Injected 0.5 mgm. histamine hydrochloride (intramuscularly)		
12:30	12.8	0.38	0.46
1:00	7.6	0.46	0.53
1:40	0.5	Present not determined	

Two other experiments on the same animal yielded similar results. Dog VI (gastric fistula) also gave definite secretion after similar generally toxic doses of atropine (3.5 mgm. per kilo body weight). It is further interesting to note that the histamine always increased the general toxic symptoms already produced in the animal by the atropine, rather than neutralizing them.

*Relative efficacy of food and histamine stimulation.* In a previous section it has been shown that a food stimulation can be completely inhibited by 1 mgm. of atropine, and yet we have just seen that 21 mgm. of atropine were not sufficient to inhibit the secretion from 0.5 mgm. of histamine. In order to test this relationship more clearly an

TABLE 6

*Effect of atropine on the stimulation produced by the intramuscular injection of 1 cc. gastrin (Sb6B). Dog IV—Pawlow stomach. The gastrin was injected three-fourths to one hour after the atropine.*

ATROPINE SULPHATE	GASTRIN	TIME	JUICE	HYDROCHLORIC ACID	
				Free	Total
mgm.	cc.		cc.	per cent	per cent
None	1	1st hour	10.1	0.32	0.36
		2nd hour	2.6	0.27	0.36
2.4	1	1st hour	3.5	0.27	0.32
		2nd hour	2.3	0.32	0.36
3.2	1	1st hour	2.9	0.27	0.32
		2nd hour	0.5	0.20	0.26
4	1	1st hour	3.0	0.16	0.23
		2nd hour	1.8	0.32	0.36

TABLE 7

*Effect of atropine sulphate on stimulation by 0.5 mgm. histamine hydrochloride both injected intramuscularly*

ATROPINE SULPHATE	HISTAMINE HYDRO- CHLORIC ACID	TIME	DOG VIII. PAWLOW STOMACH			DOG IX. PAWLOW STOMACH		
			Juice	Hydrochloric acid		Juice	Hydrochloric acid	
				Free	Total		Free	Total
mgm.	mgm.			per cent	per cent		per cent	per cent
None	0.5	1st hour	10.5	0.27	0.38	21.8	0.56	0.60
		2nd hour	1.0	0.15	0.22	2.6	0.37	0.41
0.025	0.5	1st hour	6.0	0.36	0.44	16.2	0.48	0.58
		2nd hour	0.6	0.04	0.01	2.2	0.29	0.41
0.05	0.5	1st hour	0.5	0.27	0.33	10.0	0.38	0.42
		2nd hour	0.1			1.0	0.36	0.40
0.1	0.5	1st hour	2.0	0.18	0.32	7.0	0.37	0.50
		2nd hour	1.0	0.25	0.30	0.2		
0.2	0.5	1st hour	2.1	0.19	0.23	6.5	0.41	0.46
		2nd hour	0.5	0.23	0.31	0.6	0.44	0.48

experiment was run in which the secretion induced by food was inhibited by atropine and then started by histamine.

*Dog VIII. Pawlow stomach*

TIME	JUICE	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	per cent	per cent
9:50	Dressed		
10:50	1.4		
11:00	Ate standard meal		
12:00	2.0	0.00	0.07
1:00	13.0	0.30	0.38
1:10	Injected 0.5 mgm. atropine sulphate (intramuscularly)		
1:40	1.5	0.23	0.30
2:10	0.4	Trace not estimated	
2:15	Injected 0.5 mgm. histamine hydrochloride (intramuscularly)		
2:45	7.8	0.43	0.47
3:25	3.8	0.44	0.48
3:45	2.2	0.39	0.42
4:30	4.0	0.40	0.45

An experiment run on the same animal two days later showed the period of inhibition for 0.5 mgm. of atropine sulphate under food stimulation to be approximately three hours and other experiments have shown that injections of histamine hydrochloride falling on an empty stomach rarely produce secretion for a period of over one and one-half hours. With these facts before us the examination of the protocol above shows that the atropine inhibited the food secretion, that the histamine stimulated the mechanism immediately, in its usual fashion, and that just as the results of the latter stimulation came to a standstill the food secretion reasserted itself.

#### DISCUSSION

*Qualitative changes in gastric juice under atropine injection.* Our findings on the effect of atropine on secretion under food stimulation agree well with those of Riegel (4) for the stomach and of Bylina (5) for the pancreas. The latter has studied the question more completely and he finds a reduction in quantity of juice, acid, total nitrogen, enzyme and dried residue. Our results show that the pepsin and quantity output are first affected. The concentration of acid may be maintained until the quantity has been reduced 50 per cent to 80 per cent, after which it in turn rapidly decreases. Attention has already

been called to the observation that with rapid increase in the quantity of juice secreted by a normal stomach the acid keeps pace and is able to maintain itself at a high concentration. On the other hand the pepsin partition lags behind to such an extent that its concentration in reality becomes quite low. Further, one of the authors (6) has previously shown that in parathyroid tetany a juice of fairly high acid content and extremely low peptic activity is frequently secreted. All of these observations suggest that the mechanisms of the acid and pepsin secretion are not the same, and that under different states of the gastric mucosa, which may occur in the course of a normal digestion period or be induced experimentally, these mechanisms may respond differently to the same stimulus.

*Site of action of atropine and gastrin bodies.* A quantitative antagonistic relation appears to exist between atropine on one hand and gastrin and histamine on the other. With a fixed small dose of gastrin or histamine the secretion can be practically inhibited by raising the dose of atropine. However, generally toxic doses of atropine which are short of the lethal point do not completely inhibit secretion when the gastrin dosage is increased. Secretion can therefore always be obtained by these bodies after atropine.

Certain generalizations have grown out of the use of atropine in the study of secretory mechanisms. One generalization is to the effect that atropine inhibits secretion originating from nervous mechanisms, but not that from a chemical or humoral origin. In the case of the salivary glands it has been shown that after atropine the chorda tympani is no longer able to excite secretion, but sympathetic stimulation usually causes a flow of saliva. This localizes the action of atropine to the terminations of the chorda lingual nerve. These studies were, however, carried out under ether anesthesia.

Bayliss and Starling (7) state that it is impossible to paralyze the flow of pancreatic juice produced by secretin by any dose of atropine although they do not specifically state what dosages were used. They, too, worked under ether anesthesia.

Bylina (5) found that 0.005 gram atropine reduced, but did not abolish the flow of pancreatic juice obtained by introducing HCl into a duodenal fistula, and neutral oil and sodium oleate into stomach. In other experiments he drained the gastric juice from a fistula so that none was escaping into the duodenum and found a spontaneous fasting secretion of the pancreas which was changed and reduced, but not abolished by a similar dose of atropine. He reviews the literature

rather carefully and emphasizes the necessity of working with chronic fistulae and without the use of ether. He interprets all these findings to mean that the nervous element in secretion is involved to the extent to which a reduction in secretion has occurred.

Von Anrep (8) working with decerebrated dogs found that atropine paralyzes the secretory but not the inhibitory fibers in the vagus going to the pancreas. This effect is not a vascular one, for careful measurement of blood flow through the gland shows that the vagi carry no vasomotor fibers. The inhibitory fibers he believes are really motor fibers to the ducts which analogy suggests should be affected by the atropine also.

Mathews (9) studying the secretion of saliva after clamping the blood supply to the gland emphasized the view that the action of atropine is rather on the cell than on the terminations in the chorda tympani. He presented no conclusive evidence supporting this view, but insisted its adoption was a more plausible explanation of the facts in hand.

Popielski (10) observed that the decreased coagulability of the blood drawn from the vena sub-maxillaris interna as brought about by stimulation of the chorda tympani was again increased in coagulability by the injection of 15 cc. of 0.1 per cent atropine sulphate solution. Further, that if at the time of the most marked atropine effect the chorda is again stimulated the increased coagulability remains and we have no flow of saliva. Similarly he found atropine to increase the coagulability of blood rendered less coagulable by the injection of barium chloride or pilocarpine. However the decreased coagulability and the secretion of saliva brought about by stimulation of the sympathetic nerves to the salivary gland is not overcome by atropine.

We certainly are far from knowing the site of action of atropine. The whole subject needs reinvestigation. We can at least say that while the terminations of the vagi may be affected by atropine they are not the sole site upon which the drug is acting in reducing the secretion under histamine or gastrin stimulation. Our previous studies have shown that doubly vagotomized animals give excellent secretion following the injection of these substances. There remains to be considered the nervous element in Auerbach's plexus and the cell itself. So far as histamine and gastrin are concerned the decrease in secretion is a uniform reciprocal of the dose of atropine. The curve is a uniform one with no irregularities. The food stimulation is very readily inhibited by atropine but subsequent injections of histamine showed

the mechanism to be still quite sensitive to this excitant. Further, there appears to be one irregularity, in its curve of decrease; the acid concentration is well maintained after the quantity and peptic partitions have suffered seriously.

We therefore suggest that in the ranges within which atropine paralyzes the food secretion, its point of action is largely nervous (terminations of vagi and Auerbach's plexus); in larger doses (1 mgm.) of atropine sulphate its action must be largely on the gland cells. If the physiological secretion of juice is in any way dependent on substances such as histamine and gastrin, then we would suggest that these bodies may be liberated under nervous influences. Once liberated they become the effective agent in arousing the gastric cell directly to activity. Take as an illustration dog IX which secreted 15 to 18 cc. of juice an hour on a standard meal, a secretion which was inhibited completely by 0.5 mgm. of atropine. The same animal secreted 21 cc. of juice on 0.5 mgm. of histamine. After the same dose of atropine (0.5 mgm.) the quantity still amounted to 14 cc. per hour. It is easy to see how the secretion might be blocked at the site of the nerves with their lower threshold of sensitivity to atropine.

The general toxic effect of atropine may be expressed in nervous depression or exaltation, restlessness and vomiting. All of these symptoms indicate that the atropine effect is now on the general body cells. Toward this general poisoning the gastric cells appear to be neither more sensitive nor more resistant. The evidence of their poisoning is found in their progressive failure to respond to stimulation. Thus the lethal dose (for the animal) of atropine becomes the one of complete inhibition for gastrin bodies. This conforms with our findings that an inhibitory dose to food stimulation is not a generally toxic one. However, when once these chemical excitants (gastrin bodies) are liberated then only a generally toxic dose of atropine, which abolishes not only the secretion of the gastric glands but also modifies all other protoplasmic activities, must be used before the secretion can be arrested.

If this view should prove correct we have the analogy with the pancreas as follows: Pancreatic secretin originates in the duodenal mucosa at a point where the effective stimulus (HCl) is normally present. Much discussion has centered about what part the nerves here play in the liberation of secretin from pro-secretin. The blood must now transfer the secretin to the pancreas. In the case of the stomach the whole mechanism is condensed within the mucosa of the stomach

for here the effective stimulus, whatever it be, can liberate the gastrin rendering it immediately available for secretory stimulation. At this point attention is called to the fact that through a rather extended series of experiments (2) we have never been able to find any gastrin bodies in the gastric juice, which is in keeping with this view.

Finally, it is evident that two such classes of drugs as histamine and gastrin on the one hand and atropine on the other have no power of neutralizing each other. The effect of combined doses is to increase rather than to diminish the toxicity on the general cells of the body using the nervous system as an index. In small doses they are possibly acting upon the secretory structures at different sites. In larger doses the antagonism is transferred to the gland cell, whose response or inhibition during viability is determined by the concentrations of atropine and gastrin bodies presented to the cell at any given unit of time.

#### CONCLUSIONS

1. A stimulation of the gastric glands with food is completely inhibited in the dog over a period of more than five hours by the intramuscular injection of 1 mgm. of atropine sulphate. This inhibitory dose is not a generally toxic one.

2. The quantity and pepsin concentration are immediately and progressively reduced by doses of atropine sulphate as small as 0.025 mgm. The acid concentration is not affected until the quantity has been reduced by an amount approximately 50 to 80 per cent of its original value.

3. Gastrin and histamine on the one hand, and atropine on the other, have a quantitative antagonistic effect on gastric secretion. By increasing the dose in either class of substances a secretion or inhibition can be obtained.

4. Gastrin and histamine stimulation occur following injections of quantities of atropine which appear to be just short of the lethal dose.

5. The antagonism does not consist of a chemical neutralization since each substance increases the general toxicity of the other for body cells if the nervous system be taken as an index.

6. The view is favored that in small doses atropine and gastrin bodies attack the secretory mechanism at different points; in larger doses the site of their action is transferred to the gland cell.

7. If gastrin and related bodies are of physiological importance it is suggested that they may be liberated by secretory nerves and that they then become the active exciting agent of the cell to secretion.



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# FURTHER OBSERVATIONS ON THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS, AND THE ACTION OF STRYCHNINE AFTER CERVICAL CORD SECTION

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In a previous paper (1) we concluded that in the cat after transection of the spinal cord in the cervical region the epinephrin output is not abolished, but is still sustained from the upper thoracic cord. In acute experiments it was shown that the previous rate of output might continue unchanged after the cervical cord transection, indicating that the mechanism in the thoracic cord was actually functioning at the moment of the cervical section. It is obvious that if there is an epinephrin-secreting center in the thoracic cord it may be subject to spinal shock after lesions of the cervical cord like other spinal centers, and that the susceptibility to shock might be expected to vary in different kinds of animals and might be influenced by the experimental conditions, especially the circulatory changes caused by the cervical lesion. In the present research, accordingly, we have employed not only cats, but also dogs and one or two monkeys and rabbits, and have eliminated the upper portions of the central nervous system in cats by ligation of their arterial supply as well as by the knife. The stimulating action of strychnine upon the central mechanisms governing the epinephrin output having become known to us since our last paper, we have made a number of experiments in which this drug was used after the cervical transection, in order to see whether the epinephrin output was increased by it, as is the case when the nervous system is intact and the epinephrin being liberated at the ordinary rate.

*Note.* By "ordinary output" or "normal output" we mean an output within the range observed by us in animals in which we have not purposely used procedures calculated to alter the output (such as the administration of drugs like

strychnine or artificial stimulation of the splanchnic nerves). In referring to this normal output we often use the phrase "under our experimental conditions," or words to that effect. If we sometimes omit this qualifying phrase it is simply because its continual repetition becomes tedious. It is quite true that as we have gone on and have seen within how relatively narrow a range the output, as measured by us, varies with different anesthetics and different operations, we have inevitably tended more to the opinion that a quantity which is not demonstrably affected by changes in such important experimental conditions must depend upon something more fundamental than the anesthesia, opening of the abdomen, etc. The fact that the output in one and the same acute experiment is sensibly the same when adrenal blood is first obtained as it is later on when additional operative procedures have been employed, suggests that what we term the ordinary or normal spontaneous output is not initiated or sustained by the trauma. Cannon (2) has affirmed that there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along the splanchnic fibers governing the adrenal secretion. "The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened." The only evidence he offers for this is the statement that opening the abdomen causes impulses to descend the splanchnic fibers to the intestines which inhibit their movements. As the adrenal secretory fibers are also sympathetic and run in the splanchnics they must be stimulated too. It would be just as logical to say that as vasoconstrictor fibers for the splanchnic area are sympathetic and run in the splanchnics they must also be stimulated and therefore there should be a marked rise of blood pressure due to splanchnic vasoconstriction on opening the abdomen. The only way of showing that opening the abdomen increases the epinephrin output is to prove it experimentally and this has not been done. The same is true of the alleged influence of anesthetics. Cannon says "evidence exists in the inhibitory influence of anesthesia on gastro-intestinal movements that anesthesia alone can arouse splanchnic impulses." And among these he obviously includes impulses passing along the splanchnics to the adrenals to increase the epinephrin output. Yet he has stated in another paper (3) that he has found anesthesia by urethane to be "in fact unattended by any increase of adrenal secretion demonstrable by our tests." Now if urethane does not increase the output, ether and morphine certainly cannot do so since we have found the output with these anesthetics less if anything on the average than with urethane. While we do not think that Cannon's work has shown anything at all as to the influence or want of influence of urethane upon the epinephrin output, and he has published no experimental data whatever upon this point, his conclusion that the employment of an anesthetic has no influence upon the results of his own experiments whereas the employment of the same anesthetic vitiates ours, is somewhat puzzling. But not only can he use urethane with impunity, while he indicates that we cannot do so, but he can open the abdomen, of course under anesthesia, and still obtain a certain reaction with asphyxia which he interprets as proving an increased output of epinephrin, whereas he says that when we open the abdomen we immediately deprive ourselves of all possibility of obtaining evidence of any such effect since we are working with an animal under an anesthetic and with opened abdomen. Cannon's suggestion that the reason we fail to obtain evidence of an increased output of epinephrin in such conditions as asphyxia is that the opening of the abdomen produces a secretion which

is "unsurpassable." This suggestion is inconsistent with his own statements that the output of epinephrin caused by stimulation of the central end of the sciatic is "from 5 to 25 times the amount regarded by Stewart and Rogoff as the normal output," and that the reaction on which he relies to demonstrate the increased output is elicited in equal intensity by asphyxia.

The results of the greater part of the experiments are condensed into three tables. Only a sufficient number of protocols to illustrate the various types of experiment or to illustrate experiments which could not be tabulated have been introduced.

#### ACUTE EXPERIMENTS ON CATS

In table 1 are shown the results of acute experiments on cats in which the epinephrin output was assayed on rabbit intestine (and uterus) segments before and after transection of the cervical cord.

In three of the cats (350, 407 and 67) the output, which was of the ordinary magnitude before the cord section, remained practically unaltered by that operation. The data on one of these animals have already been published (1), but for the sake of completeness they are included in the table. In one of these cats (407) the intravenous injection of strychnine after transection of the cord raised the output of epinephrin to seven times the initial value. As the experiment illustrates at the same time the possibility of dividing the cervical cord without reducing the epinephrin output and without interfering with the characteristic action of strychnine upon the output, the condensed protocol is reproduced.

##### *Condensed protocol. Cat 407; female; weight, 2.46 kgm.*

- 9.05 a.m. Anesthetized with ether, inserted cannulae into trachea, carotid artery and external jugular vein. Obtained a specimen of indifferent (jugular) blood.
- 9.20 a.m. Exposed cord below mid-cervical region. Made cava pocket, tying coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.
- 9.45 a.m. Cava pocket completed. Collected adrenal blood.
- 9.48½ a.m. First specimen, 1.9 gram in 30 seconds (3.8 grams per minute).
- 9.49 a.m. Second specimen, 4.85 grams in 90 seconds (3.2 grams per minute).
- 9.55 a.m. Blood pressure 103 mm. of mercury.
- 10.00 a.m. Transected cord above the origins of the 6th pair of cervical nerves (position of section was verified at autopsy). Spontaneous respiration continued; artificial respiration not employed. After cord section the pupils were about one-third dilated and eye reflexes good.

- 10.03 a.m. Blood pressure 50 mm. of mercury.  
10.05 a.m. Third adrenal specimen, collected for 30 seconds (not weighed).  
10.05½ a.m. Fourth adrenal specimen, 1.85 gram in 5 minutes (0.37 gram per minute).  
10.10 a.m. Started artificial respiration.  
10.15 a.m. Blood pressure 50 mm. of mercury.  
10.17 a.m. Intravenous injection of 0.45 mgm. strychnine: within ½ minute after strychnine injection a tonic spasm occurred for a few seconds and this was followed by clonic convulsions which recurred intermittently during the rest of the experiment.  
10.20 a.m. Fifth adrenal specimen, collected for 30 seconds (not weighed).  
10.20½ a.m. Sixth adrenal specimen, 2.3 grams in 10 minutes (0.23 gram per minute).  
10.33 a.m. Blood pressure 30 mm. of mercury. Another specimen of indifferent blood was now obtained. Combined weight of adrenals, 0.375 gram. (There was about 75 per cent of serum in the bloods).

As it is impossible to reproduce all of the tracings used for the assay even in one experiment, we shall illustrate this one typical experiment more completely than the rest, for which it must serve as a sample of the manner in which we arrive at the epinephrin concentrations. Some of the tracings are reproduced in figures 1 to 4.

The 2nd specimen was found to be decidedly weaker than 1:4,000,000 adrenalin (fig. 1, confirmed by other observations), decidedly stronger than 1:8,000,000, somewhat stronger than 1:6,660,000, somewhat weaker than 1:5,300,000. It was taken at 1:6,000,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00021 mgm. per kgm. per minute.

The 4th specimen, collected after section of the cord, was much stronger than 1:1,300,000 (fig. 2), not very different from 1:660,000 (fig. 3). Another observation indicated that it was probably somewhat weaker than 1:660,000. It was distinctly weaker than 1:530,000 (fig. 3). It was taken at 1:700,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00021 mgm. per kgm. per minute.

The concentration of epinephrin in the 6th adrenal specimen, obtained after injection of strychnine, was so great that in order to make a good assay it was necessary to dilute it with indifferent blood. In this way it was shown to be much stronger than 1:165,000 adrenalin (fig. 4). The 6th specimen diluted with 3 volumes of indifferent blood was much stronger than 1:660,000 adrenalin. Diluted with 7 volumes of indifferent blood it was still found to be much stronger than 1:1,000,000, i.e., the 6th specimen was much stronger than 1:125,000.

TABLE 1  
Acute experiments. Cats

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD										REMARKS
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute	For animal	Per kilogram	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute	For animal	Per kilogram	mgm.	
320	3.15	v-vi	0.295	3.25	300	0.65	40	1:6,000,000	0.00011	0.000035	4.4	330	0.8	44	1:6,000,000	0.00013	0.00004				Shock on cord exposure: 80 cc. Ringer injected before collection of each adrenal specimen.
321	3.89	above iv	0.922	2.75	360	0.46		1:2,700,000	0.00017	0.000045	2.05	720	0.17		1:1,000,000	0.00017	0.000045				Without injection of Ringer With Ringer injection, 100 cc.
322	2.25	v-vi	0.487	7.0	1203	3	144	1:4,500,000	0.0007	0.0003	3.4	720	0.30	58	1:800,000	0.0004	0.00017				
384	2.63	above vi	0.275	4.95	1202	5	117	1:3,500,000	0.0007	0.00027	7.1	90	4.7	46	1:9,400,000	0.0005	0.0002				After cord section the blood pressure fell to 32 mm. of mercury. 100 cc. Ringer injected and circulation improved.

399	2.69	v-vi	0.268	5.3	903.5	110	1:5,000,000	0.00085	0.00025	1.5	3300.3	471:1,500,000	0.0002	0.00075	0.5 mgm. strychnine injected before collection of this specimen.
										1.8	2400.45	401:250,000	0.0018	0.0007	
406	2.09	vi-vii	0.474	3.75	1201.87	112	1:4,600,000	0.0004	0.00024	3.15	1201.57	861:6,000,000	0.00025	0.00012	After cord section blood pressure fell to 26 mm. 60 cc. Ringer injected and blood pressure rose to 86 mm. of mercury.
										1.15	3600.2	401:150,000	0.0013	0.0006	0.6 mgm. strychnine injected before this specimen.
407	2.46	above vi	0.375	4.85	903.2	103	1:6,000,000	0.00053	0.00021	1.85	3000.37	501:700,000	0.00053	0.00021	0.45mgm. strychnine injected before this specimen.
										2.3	6000.23	301:70,000	0.0033	0.0014	
418	1.96	v	0.232	(a)3.2 (b)3.35	902.1 1201.7	105 94	1:4,000,000 1:1,700,000	0.0005 0.0001	0.00025 0.0005	1.0	6000.1	241:100,000	0.001	0.0005	0.25 mgm. strychnine injected after (a). Blood pressure rose to 120 mm. Hg. Cord section after strychnine injection.
67	2.31	above v	0.440	10.0	1205.0		1:13,000,000	0.0004	0.00017	2.1	4300.3	1:800,000	0.0004	0.00017	

TABLE 1—Continued

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD							REMARKS
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	For animal	Epinephrin output per minute	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	For animal	Epinephrin output per minute	
324	2.1		0.367	5.55	180	1.85		1:9,000,000	0.0002	0.0001	4.75	300	0.95		1:4,000,000	0.00024	0.0001	Bulb and brain eliminated by ligation of head arteries.
32	2.05		0.252	5.15	180	1.72		1:2,500,000	0.0007	0.00035	2.3	300	0.5		1:675,000	0.0007	0.00035	Bulb and brain eliminated by ligation of head arteries.
435	2.5		0.314	1.9	150	0.76		1:800,000	0.00095	0.00038	2.5	180	0.83		1:700,000	0.0012	0.00048	Bulb and brain eliminated by ligation of head arteries.
383	3.98	v-vi	0.602	8.05	90	5.4	129	1:5,400,000	0.001	0.00025	4.45	180	1.48	93	1:3,000,000	0.0005	0.00013	Both adrenals discharging in all specimens.
385	2.75	iv	0.612	6.2	180	2.08	114	1:2,900,000	0.0007	0.00025	3.25	240	0.81	40	1:1,000,000	0.0008	0.0003	Right adrenal only discharging in both specimens.

\* In these two animals the spinal operation was a semisection, left in 383, and right in 385.



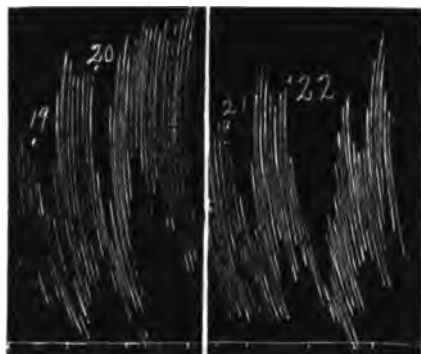


Fig. 1. Intestine tracings. Bloods from cat 407. At 19 and 21 Ringer was replaced by indifferent (venous) blood and this at 20 by the 2nd adrenal specimen (collected before cervical cord section); at 22 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). As in all the figures the time trace is in half-minutes. Reduced to one-half.

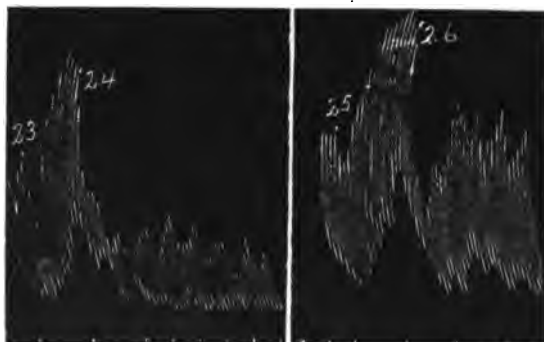


Fig. 2. Intestine tracings. Bloods from cat 407. At 23 and 25 Ringer was replaced by indifferent (venous) blood and this at 24 by the 4th adrenal specimen (collected 5½ minutes after cervical cord section); at 26 by indifferent blood to which was added adrenalin to make a concentration of 1:1,300,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-sevenths.

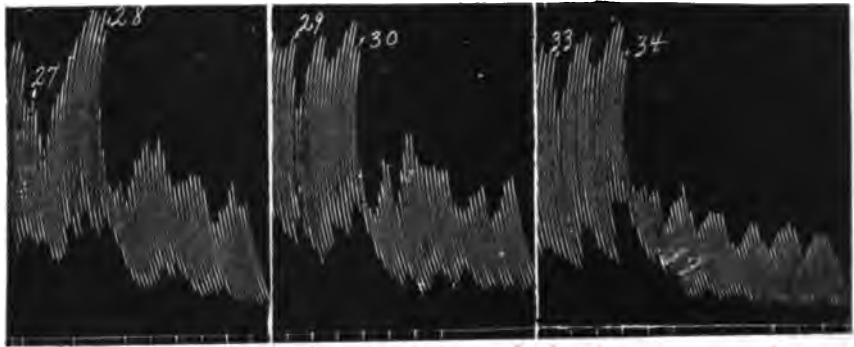


Fig. 3. Intestine tracings. Bloods from cat 407. At 27, 29 and 33 Ringer was replaced by indifferent (venous) blood and this at 28 by indifferent blood to which was added adrenalin to make a concentration of 1:660,000; at 30 by the 4th adrenal specimen (collected 5½ minutes after cervical cord section); at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:530,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-sevenths.

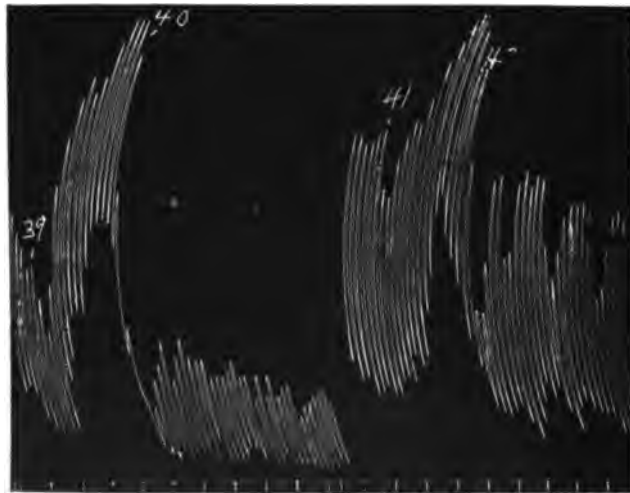


Fig. 4. Intestine tracings. Bloods from cat 407. At 39 and 41 Ringer was replaced by indifferent (arterial) blood and this at 40 by the 6th adrenal specimen (collected 3 minutes after intravenous injection of strychnine and 10 minutes after cervical cord section) diluted with 3 volumes of indifferent blood; at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

In the same way it was shown that the specimen did not differ much from 1:66,000, being probably a little weaker. This was confirmed by numerous observations on the adrenal blood diluted with 7 volumes of indifferent blood, and also on the adrenal blood diluted with 14 volumes of indifferent blood. The 6th specimen was finally taken at 1:70,000, corresponding to an output of 0.0033 mgm. per minute for the cat, or 0.0014 mgm. per kgm. per minute.

In two of the cats in table 1 (320 and 321) the output was already much below the ordinary average before the cord was cut. These were two of the earliest experiments, and at this time we were in the habit of passing a thread under the cord after exposure. The thread was not tied, of course, but was used to make sure that the transection was complete. Later on the section was made intradurally through a longitudinal slit in the dura by means of small blunt pointed scissors, and the cord was not disturbed before collection of the initial adrenal specimen, apart from being laid bare at the level where it was to be divided. Blood vessels lying between the cord and the walls of the vertebral canal escaped injury except, of course, where the bone was removed. In cats 320 and 321 the low initial output of epinephrin was associated with a low blood pressure. Transection of the cord left the output unaltered. The condensed protocol of cat 321 follows.

*Condensed protocol. Cat 321; male; weight, 3.89 kgm.*

- 8.30 a.m. Urethane, 6 grams per stomach tube.
- 9.00 a.m. Inserted tracheal cannula, exposed cord in mid-cervical region and placed a loose ligature under the cord. During the manipulations around the cord the cat stopped breathing and the circulation became feeble. Artificial respiration was at once started. Obtained a specimen of indifferent blood (femoral vein). Made cava pocket.
- 10.06 a.m. Pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above the bifurcation) were tied in addition to the veins entering the cava pocket. Collected adrenal blood.
- 10.10 a.m. First specimen, 0.9 gram in 1 minute.
- 10.11 a.m. Second specimen, 2.75 grams in 6 minutes (0.46 gram per minute).
- 10.20 a.m. Transected cord just above the origins of the 4th pair of cervical nerves.
- 10.23 a.m. Third adrenal specimen collected for 1 minute (not weighed).
- 10.24 a.m. Fourth adrenal specimen, 2.05 grams in 12 minutes (0.17 gram per minute).
- 10.35 a.m. Intravenous injection of 50 cc. Ringer.
- 10.45 a.m. Intravenous injection of 50 cc. Ringer.
- 10.50 a.m. Fifth adrenal specimen, collected for 1 minute.
- 10.51 a.m. Sixth adrenal specimen, 2.55 grams in 6 minutes (0.425 gram per minute). Another specimen of indifferent blood was now obtained from the right heart. Combined weight of adrenals 0.922 gram.

We have had many illustrations in this work of a fact which may have some general bearing upon the mechanism of spinal shock but whose bearing upon that depression of the spinal mechanism concerned in the liberation of epinephrin sometimes observed after cervical cord transection, and which we suggest is a spinal shock phenomenon, seems incontestable. When the blood pressure and therefore the blood flow through the adrenals are lowered very considerably while the central nervous system is intact, we always find a corresponding increase in the epinephrin concentration in the adrenal vein blood, which until the possible maximum concentration has been attained keeps the output of epinephrin per unit of time as high as the initial output, at any rate for the relatively short duration of the low blood pressure which we have studied. That is to say, with intact nervous system the epinephrin secreting mechanism in the thoracic cord continues to sustain the original output in spite of the impaired circulation. It is, of course, to be supposed that after the low blood pressure has endured for a considerable time (vascular shock) the output will be diminished. But this point is not easily reached in experiments of moderate duration. When, however, injury to the cervical cord is associated with a low blood pressure, which would not of itself cause diminution of the epinephrin output, the output may be markedly diminished and then a slow adrenal blood flow may be accompanied by a great *decline* in the concentration of epinephrin in the adrenal vein blood.

It has often been pointed out that spinal shock cannot be due to the fall of blood pressure since this affects equally the parts of the central nervous system above and below the lesion. No doubt in a general way this is true enough. But if attention be paid to the fact that the direction of flow of the blood in the main spinal arteries is caudad and the direction of flow of a good deal of the venous blood cephalad, it may appear that for some distance below a spinal transection, especially perhaps in the cervical region, the possibility would exist of a greater interference with the circulation below the lesion, for a given fall of blood pressure, than above it, in spite of the lateral anastomoses. In any case, it seems to follow pretty clearly from our observations on cats that the rupture of the upper paths is more likely to cripple the epinephrin secretory mechanism in the thoracic cord if accompanied by an unusually low blood pressure than when the circulation remains relatively efficient after the cervical cord lesion. The loss of impulses from above is the same in both cases, but the well nourished thoracic mechanism goes on sustaining the epinephrin output at a high or at the

original level, whereas the poorly nourished mechanism in the absence of those impulses is unable to do this.

In cats 322, 399, 406 (table 1) the output was diminished after transection of the cord to one-half to one-third of the initial output. When strychnine was given, in two of the animals in which the output had been diminished by the cord section, it was raised not only to the initial value but to three times that amount. Included in table 1 are three experiments in which the upper parts of the central nervous system were eliminated by permanent ligation of the innominate, left subclavian and other arteries supplying the head with blood. There could not be any doubt that at the time of collection of the adrenal blood after ligation of the arteries the elimination of the bulb and everything above it was complete. The eye reflexes had long since disappeared, the respiratory center had ceased to discharge, the swallowing center had become inactive, and so on. The observations of Stewart, Guthrie, Burns and Pike (4) show that after such ligation of arteries in cats there is total elimination of the bulb and everything above it and some interference with upper cervical segments.

In these animals, without exception, the epinephrin output after elimination of the bulb and everything above it by the bloodless method was not at all diminished. It is clear that by this method the infliction of direct injury upon the portion of the cord concerned in sustaining the epinephrin output by such gross interference with its circulation as might be caused by subdural hemorrhage, cutting and blocking of spinal arteries and veins, is largely obviated. The residual blood pressure after elimination of the brain and bulb is probably also in general somewhat higher than when the cervical cord is divided by the knife, and the proportion of experiments in which the output continued without diminution is increased. Two of the protocols follow.

*Condensed protocol. Cat 325; male; weight, 2.05 kgm.*

- 9.20 a.m. to 9.50 a.m. Anesthetized with ether, inserted tracheal cannula, placed ligatures around the innominate, left subclavian and both carotid arteries, but did not tie off yet, started artificial respiration (pleura punctured).
- 10.10 a.m. Cava pocket completed; coeliac axis, renal, mesenteric arteries and abdominal aorta (above bifurcation) tied in addition to the veins entering the cava pocket. Collected adrenal blood.
- 10.14 a.m. First specimen, 1.2 gram in 30 seconds (2.4 grams per minute).
- 10.14½ a.m. Second specimen, 5.15 grams in 3 minutes (1.72 gram per minute).
- 10.22 a.m. Tying off head arteries completed.

- 10.23 a.m. Pupils maximally dilated. No eye reflexes present. Gums pale. Intra-ocular pressure diminished. No spontaneous respiratory movements.
- 10.26½ a.m. Third adrenal specimen, 0.6 gram in 30 seconds (1.2 gram per minute).
- 10.27 a.m. Fourth adrenal specimen, 2.3 grams in 5 minutes (0.5 gram per minute).
- 10.32 a.m. No change in symptoms showing complete cerebral anemia and no change observed thereafter throughout the rest of the experiment. Obtained (indifferent) venous blood. Combined weight of adrenals, 0.252 gram.
- Autopsy.* All of the head arteries were tied off (i.e., innominate, left subclavian, proximal to the vertebrals and both carotids).

The 6th specimen was shown to have a higher concentration than the 4th. As the concentration of the 4th specimen was 1:675,000, the 6th must have had a maximal concentration, although of course with the slow flow a full calculated output could not be expected.

*Condensed protocol. Cat 435; female (pregnant); weight 2.5 kgm.*

- 10.15 to 10.45 a.m. Anesthetized with ether, inserted tracheal cannula, placed ligatures around the innominate, left subclavian (proximal to origin of vertebral), and both carotid arteries, but did not tie them off.
- 11.00 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) tied, in addition to the veins entering the cava pocket. Collected adrenal blood.
- 11.04 a.m. First specimen, 0.6 gram in 30 seconds (1.2 gram per minute).
- 11.04½ a.m. Second specimen, 1.9 gram in 2½ minutes (0.76 gram per minute).
- 11.08 a.m. Cat lightly anesthetized; pupils contracted; eye reflexes good; started tying cerebral vessels.
- 11.10 a.m. Head arteries tied. Pupils widely dilated and eye reflexes gone. Cat still gasping.
- 11.12 a.m. Mucosa of mouth and nose pale. Cat still gasps occasionally.
- 11.14 a.m. Hemostat clamped on left subclavian proximal to ligature.
- 11.15 a.m. No gasps; all upper reflexes gone, tail and hind leg reflexes still present, notwithstanding ligation of vessels in making cava pocket; intra-ocular tension low; swallowing reflex gone. A nick in nasal septum does not bleed.
- 11.25 a.m. Third adrenal specimen, 0.8 gram in 30 seconds (1.6 gram per minute).
- 11.25½ a.m. Fourth adrenal specimen, 2.5 grams in 3 minutes (0.83 gram per minute).
- 11.38 a.m. All symptoms of complete cerebral anemia still the same.
- 11.40 a.m. Fifth adrenal specimen, collected for 30 seconds.
- 11.40½ a.m. Sixth adrenal specimen, 1.65 gram in 6 minutes (0.275 gram per minute). Now tied off the adrenal veins and obtained (indifferent) venous blood from the cava. Combined weight of adrenals 0.314 gram.
- Autopsy:* Verified the complete ligation of all blood vessels going to the head.

In cat 435 the 2nd adrenal specimen, taken before ligation of the head arteries was somewhat weaker than the 4th specimen, collected 15 minutes after completion of the ligation (fig. 5, confirmed by other observations). Diluted with 3 volumes of indifferent blood it was shown to be somewhat stronger than 1:3,600,000 adrenalin, i.e., the 2nd specimen was somewhat stronger than 1:900,000. It was finally assayed at 1:800,000, corresponding to an output of 0.00095 mgm. per minute for the cat, or 0.00038 mgm. per kgm. per minute.

The 4th specimen was proved to be much stronger than 1:1,350,000, stronger than 1:900,000. Diluted with 3 volumes of indifferent blood

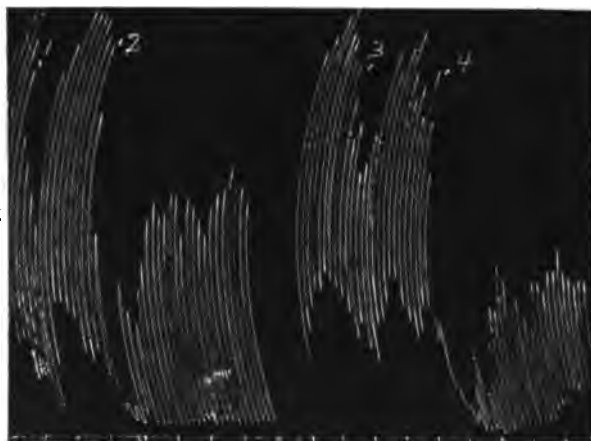


Fig. 5. Intestine tracings. Bloods from cat 435. At 1 and 3 Ringer was replaced by indifferent (venous) blood and this at 2 by the 2nd adrenal specimen (collected before tying off head arteries); at 4 by the 4th adrenal specimen (collected after tying off head arteries). All the bloods were diluted with 3 volumes Ringer. Reduced to one-half.

it was not much different from 1:2,700,000, i.e., the 4th specimen was about the same as 1:675,000. Taking it at 1:700,000 we get 0.0012 mgm. per minute as the output for the cat, or 0.00048 mgm. per kgm. per minute. In other words, the complete elimination of the bulb and brain had left the output fully as great as the initial output.

The 6th adrenal specimen, collected 15 to 20 minutes after the end of collection of the 4th specimen, that is after a period of more than half an hour of complete cerebral anemia, was diluted with 3 volumes of indifferent blood before assay. So diluted it was found to differ little in con-

centration from 1:1,800,000 adrenalin, being if anything a little stronger i.e., the 6th specimen was equivalent to about 1:450,000 adrenalin. It was decidedly weaker than 1:325,000. Taking it at 1:450,000 we get an output of 0.00061 mgm. per minute for the cat, or 0.00024 mgm. per kgm. per minute. The high concentration shows that at this time the adrenal secretion was still quite efficient in the absence of the bulb and brain. With the slow blood flow it could not of course be expected that a full output could be calculated out from such a concentration.

Two experiments are included in table 1 in which the cervical cord was semisectioned (cats 383 and 385). In one of these (383) after the semisection the output was reduced to about one-half the initial volume. This cannot be interpreted as indicating that the semisection diminished or abolished the output from the corresponding adrenal, but rather that the cord lesion caused a depression in the output from the two adrenals (spinal shock). For in the other cat (385), division of the right half of the cord did not interfere at all with the output of epinephrin from the right adrenal. In this cat the left adrenal was clipped off during collection of the blood, both before and after the semisection.

In addition to the cats included in table 1 in which segment assays were made of the adrenal blood, the epinephrin output was estimated by the reaction upon the pupil (sensitized by previous removal of the superior cervical ganglion) in two more cats (350 and 353). The condensed protocols follow.

*Condensed protocol. Cat 350; male, weight 1.88 kgm.*

Left superior cervical ganglion excised 13 days prior to experiment. Left pupil contracted and nictitating membrane forward.

9.00 a.m. Urethane, 4 grams by stomach tube.

10.00 a.m. Left pupil  $\frac{1}{2}$ , right pupil  $\frac{1}{2}$  maximal, both nictitating membranes retracted.

10.00 to 10.20 a.m. Inserted tracheal cannula and exposed cord in mid-cervical region. On exposing cord the left pupil became maximally dilated, the right pupil remained slit-like, both nictitating membranes were retracted. In a few minutes the left pupil came down to  $\frac{1}{2}$  maximal dilatation.

10.22 a.m. Started artificial respiration and made "long" pocket, tying the coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.

10.40 a.m. Pocket completed. Right pupil slit-like, left pupil  $\frac{1}{2}$  dilated, both nictitating membranes slightly forward, the right more than the left.



- 10.52 to 11.08 a.m. Two observations showed that a 2-minute pocket was equal to 1.0 cc. of 1:1,500,000 adrenalin, corresponding to an output of 0.00035 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute.
- 11.15 a.m. Transected cord through the origins of the 5th pair of cervical nerves (position of cord section was verified at autopsy).
- 11.18 to 11.26 a.m. Two pocket observations showed a 2-minute pocket to correspond to 1.0 cc. of 1:1,500,000 adrenal, i.e., the output was the same as before cord section. Combined weight of adrenals, 0.41 gram.

*Condensed protocol. Cat 353; old male; weight, 3.13 kgm.*

Left superior cervical ganglion excised 19 days prior to experiment. Left pupil contracted and nictitating membrane forward.

- 9.15 a.m. Urethane, 4 grams by stomach tube.
- 10.20 a.m. Urethane, 2 grams by stomach tube.
- 10.45 a.m. Exposed cord in mid-cervical and mid-dorsal regions. Loose ligatures passed under cord.
- 11.20 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above the bifurcation) were tied in addition to the veins entering the cava pocket.
- 11.22 a.m. Right pupil slit-like, left pupil  $\frac{1}{2}$  dilated, both nictitating membranes forward.
- 11.23 to 11.41 a.m. Three observations showed that a 2-minute pocket was equal to 0.5 cc. of 1:600,000 adrenalin, corresponding to an output of 0.00037 mgm. per minute for the cat, or 0.00012 mgm. per kgm. per minute.
- 11.45 a.m. Transected cord just above the origins of the 5th pair of cervical nerves.
- 11.48 a.m. to 12.00 m. Three observations showed that a 2-minute pocket was fully equivalent to 0.5 cc. of 1:1,000,000 adrenalin, corresponding to an output of fully 0.00025 mgm. per minute for the cat, or 0.00008 mgm. per kgm. per minute. Combined weight of adrenals, 0.705 gram.

#### ACTION OF STRYCHNINE AFTER CERVICAL CORD TRANSECTION

As already stated, in a number of the cats strychnine was injected after the cervical transection in order to see whether the stimulating action exerted by it upon the epinephrin output in animals with intact cord would be developed after elimination of the upper parts of the central nervous system. This was found to be the case. Increases in the output both relatively and absolutely as great as those seen with intact cord were obtained. In one of the experiments already discussed (cat 407) the initial output remained unchanged after the cord

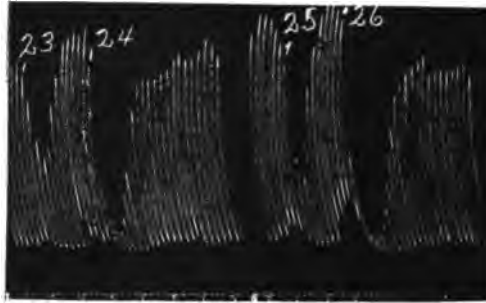


Fig. 6. Intestine tracings. Bloods from cat 406. At 23 and 25 Ringer was replaced by indifferent blood and this at 24 by the 4th adrenal specimen (collected 14 minutes after cervical cord section) and at 26 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

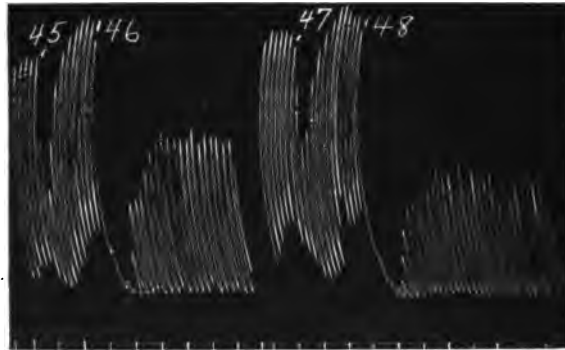


Fig. 7. Intestine tracings. Bloods from cat 406. At 45 and 47 Ringer was replaced by indifferent (venous) blood and this at 46 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000, and at 48 by the 6th adrenal specimen (collected 5 minutes after intravenous injection of strychnine and 33 minutes after cervical cord section) diluted with 4 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

section and was increased sevenfold by strychnine. In another experiment (cat 406) the output was diminished to something more than half the original output by the cervical transection and was brought up to three times the initial output by strychnine.

Figures 6 to 8 illustrate the assay in cat 406. The 2nd adrenal specimen was found to be much stronger than 1:5,300,000, somewhat stronger than 1:4,000,000 (confirmed by a number of observations), weaker than 1:2,750,000 (confirmed by a number of observations). It was finally taken at 1:3,600,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00024 mgm. per kgm. per minute.

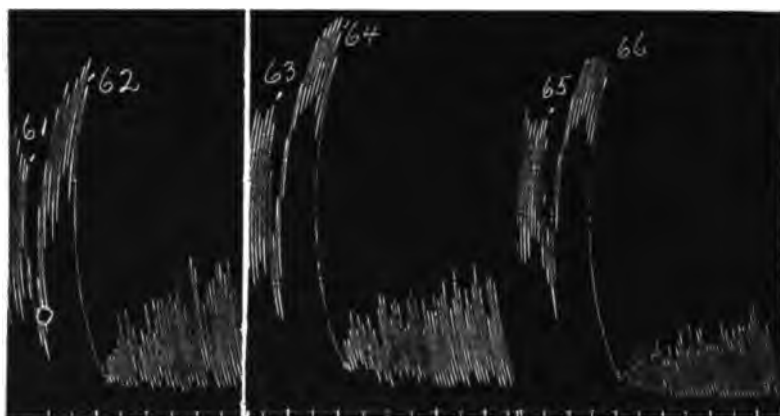


Fig. 8. Intestine tracings. Bloods from cat 406. At 61, 63 and 65 Ringer was replaced by indifferent (venous) blood and this at 62 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000; at 64 by the 6th adrenal specimen (collected 5 minutes after intravenous injection of strychnine and 33 minutes after cervical cord section) diluted with 9 volumes of indifferent blood; at 66 by indifferent blood to which was added adrenalin to make a concentration of 1:665,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

The 4th specimen, collected after transection, was weaker than 1:4,000,000 (fig. 6, confirmed by another set of observations), stronger than 1:8,000,000, somewhat stronger than 1:6,600,000. It was taken at 1:6,000,000, corresponding to an output of 0.00026 mgm. per minute for the cat, or 0.00012 mgm. per kgm. per minute.

The 6th specimen, collected after injection of strychnine, had so high a concentration of epinephrin that it was diluted with indifferent blood before assay. Diluted with 4 volumes of indifferent blood it was much stronger than 1:2,750,000, i.e., the 6th specimen was much stronger than 1:550,000. It was similarly found to be stronger than 1:265,000 (fig. 7), and slightly weaker than 1:132,000. In another dilution (with 9 volumes of indifferent blood) it was shown to be stronger than 1:275,000, decidedly weaker than 1:66,000, and not very different from 1:132,000 (fig. 8). The segment was more sensitive at this stage and still greater dilution would have been necessary for the best results. The 6th specimen was finally taken at 1:150,000, corresponding to 0.0013 mgm. per minute for the cat, or 0.00062 mgm. per kgm. per minute.

In a previous paper (5) it was shown that the action of strychnine is a lasting one. This has been illustrated in an interesting fashion in the cord transection experiments, for it has been proved that the excitation of the mechanism on which the strychnine increase in epinephrin output depends, once induced, can survive transection of the cervical cord.

*Condensed protocol. Cat 418; female; weight, 1.96 kgm.*

- 9.10 a.m. Anesthetized with ether, inserted cannulae into trachea, carotid artery and external jugular vein. Obtained a specimen of indifferent (jugular) blood.
- 9.30 a.m. Exposed cord in mid-cervical region. Made cava pocket, tying coeliac axis, renal and superior mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.
- 10.05 a.m. Cava pocket completed. Collected adrenal blood.
- 10.07½ a.m. First specimen, 1.05 gram in 30 seconds (2.1 grams per minute).
- 10.08 a.m. Second specimen, 3.2 grams in 90 seconds (2.1 grams per minute).
- 10.10 a.m. Blood pressure 105 mm. of mercury.
- 10.14 a.m. Started artificial respiration (cat breathing well spontaneously).
- 10.17 a.m. Intravenous injection of 0.25 mgm. strychnine. Clonic spasms occurred within a few seconds and the blood pressure rose to 120 mm. of mercury.
- 10.21½ a.m. Third adrenal specimen, 0.65 gram in 30 seconds (1.3 gram per minute).
- 10.22 a.m. Fourth adrenal specimen, 3.35 grams in 2 minutes (1.7 gram per minute).
- 10.24 a.m. Blood pressure 94 mm. of mercury.
- 10.30 a.m. Transected cord through origins of 5th pair of cervical nerves (position of cord section verified at autopsy).

- 10.33 a.m. Blood pressure 24 mm. of mercury. Reflexes exaggerated.  
10.34 a.m. Fifth adrenal specimen collected for one minute (not weighed).  
10.35 a.m. Sixth adrenal specimen 1.0 gram in 10 minutes (0.1 gram per minute).  
Another specimen of indifferent blood was now obtained. Combined weight of adrenals, 0.232, gram.

In figures 9 and 10 are reproduced a small sample of the tracings used in the assay in cat 418.

The 2nd specimen, obtained before injection of strychnine, was found to be stronger than 1:4,500,000 adrenalin, decidedly weaker than 1:3,000,000 and somewhat weaker than 1:3,750,000. It was assayed at 1:4,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute.

The 4th specimen, collected after administration of strychnine, but before transection of the cord, was much stronger than 1:3,000,000, somewhat weaker than 1:1,500,000 (fig. 9, confirmed by other observations). It was taken at 1:1,700,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kgm. per minute, double the initial output.

The 6th specimen, collected after section of the cord, had a very high concentration of epinephrin. It was diluted with 3 volumes of indifferent blood before assay. Thus diluted it was stronger than 1:750,000 adrenalin, i.e., the 6th specimen was stronger than 1:185,000 (fig. 10, confirmed by other observations). It was probably somewhat weaker than 1:94,000 and was taken at 1:100,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kgm. per minute.

Since the output, increased by strychnine, was not changed by the cervical transection it follows that in this animal the influence of strychnine in increasing the epinephrin output was, within the limits of error of the assay, exerted exclusively on the portion of the cord below the transection. It is of interest that in spite of the low blood pressure which followed the transection (to 24 mm. of mercury), spinal shock of the epinephrin secretory mechanism in the thoracic cord, as manifested by a fall in the rate of output, did not occur, the marked diminution in the rate of blood flow through the adrenals being compensated for by the great increase in epinephrin concentration. Where the mechanism has suffered from the condition which we suggest is analogous to spinal shock affecting other centers, a reduced blood flow may be accompanied by a diminished concentration of epinephrin, a combination practically never encountered under the conditions of our

experiments while the central nervous system is intact. The administration of strychnine in this experiment might be said to have constituted a prophylaxis against spinal shock so far as the epinephrin secretory mechanism is concerned.

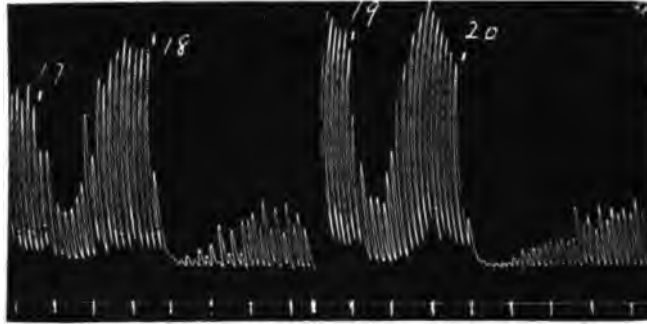


Fig. 9. Intestine tracings. Bloods from cat 418. At 17 and 19 Ringer was replaced by indifferent (venous) blood and this at 18 by the 4th adrenal specimen (collected 5 minutes after intravenous injection of strychnine); at 20 by indifferent blood to which was added adrenalin to make a concentration of 1:1,500,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

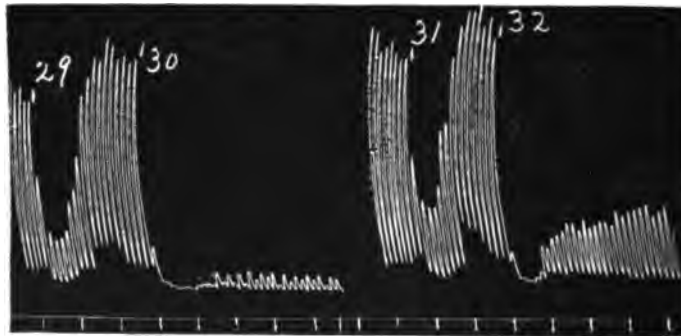


Fig. 10. Intestine tracings. Bloods from cat 418. At 29 and 31 Ringer was replaced by indifferent (venous) blood and this at 30 by the 6th adrenal specimen (collected 18 minutes after intravenous injection of strychnine and 5 minutes after cervical cord section) diluted with 3 volumes of indifferent blood; at 32 by indifferent blood to which was added adrenalin to make a concentration of 1:750,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

In contrast to the action of strychnine on animals with intact central nervous system (5) in which the maximum concentration of epinephrin was not found to surpass substantially the maximum observed without strychnine, the maximum concentration under the influence of strychnine in the cats with cervical cord transection was observed to reach extraordinarily high values (1:70,000 in cat 407; 1:100,000 in cat 418; 1:150,000 in cat 406; 1:250,000 in cat 399). This is consistent with the suggestion expressed in a previous paper (5) that the preliminary diminution in the output produced by strychnine in animals with intact central nervous system may be due to transient stimulation by the drug of a regulatory (inhibitory) mechanism situated higher up and cut off by the cervical transection.

The 2nd specimen from cat 399 (before transection) was shown on intestine segments to be decidedly stronger than 1:7,500,000, stronger than 1:6,000,000, somewhat weaker than 1:4,500,000. It was taken at 1:5,000,000, corresponding to an output of 0.00065 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute.

The 4th specimen (after transection) was much stronger than the 2nd. Diluted with 2 volumes of indifferent blood it was still stronger than 1:6,000,000, i.e., the undiluted 4th specimen was stronger than 1:2,000,000. It was found to be decidedly weaker than 1:750,000, weaker than 1:1,000,000 and not far different from 1:1,500,000, corresponding to an output of 0.0002 mgm. per minute, for the cat, or 0.000075 mgm. per kgm. per minute. Considering the small flow this is a fair output, but the striking thing is that the concentration, although increased to more than three times that of the 2nd specimen, corresponding to the diminished blood flow, is not lifted nearly to the possible maximum as would occur with intact cord, whereas when strychnine is given the concentration is increased sixfold, to much beyond the maximum seen without strychnine, while the blood flow is also increasing somewhat.

The assay on intestine segments showed that the 6th specimen (from cat 399) was much stronger than the 4th, much stronger than 1:750,000, much stronger than 1:535,000, much stronger than 1:415,000, stronger than 1:320,000, decidedly weaker than 1:180,000 and not far different from 1:250,000 adrenalin. In making these tests the adrenal blood was diluted with 2 volumes and in other observations with 4 volumes of indifferent blood. It was confirmed on the uterus (fig. 11) that the 6th specimen was much stronger than the others.

That the strychnine action is essentially a central action is best shown by administering the drug to animals from which one adrenal has been previously removed and the nerves of the other cut. Where the output has been reduced below the point of detection or, if detectable, to a small fraction of the normal average, it does not become detectable, or is not increased on injecting strychnine. On the other hand, if a substantial, although of course much reduced output, is present after the adrenal operation, either because the nerves have been incompletely divided or some regeneration has occurred after a long survival period, strychnine causes a definite increase. Illustrations of these facts have already been published in a previous paper (6).

The same thing may be proved by sectioning the adrenal nerves in acute experiments which, however, are on the whole less satisfactory on account of the fall of blood pressure produced by the denervation. A protocol from one of these experiments (cat 431) is given as an example.

*Condensed protocol. Cat 431; female; weight, 2.05 kgm.*

- 9.20 a.m. Anesthetized with ether; inserted tracheal and jugular cannulae; obtained indifferent (jugular) blood.
- 9.55 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied in addition to the veins entering the cava pocket. Collected adrenal blood.
- 9.55½ a.m. First specimen, 1.95 gram in 30 seconds (3.9 grams per minute).
- 9.56 a.m. Second specimen, 7.0 grams in 2 minutes (2.5 grams per minute).



Fig. 11. Uterus tracings. Bloods from cat 399. At 62 Ringer was replaced by the 6th adrenal blood specimen (collected 3 minutes after intravenous injection of strychnine and 23 minutes after cervical cord section) diluted with 6 volumes of indifferent blood; at 63 by indifferent (venous) blood; at 64 by indifferent blood to which was added adrenalin to make a concentration of 1:2,250,000; at 65 by the 6th specimen diluted with 4 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). At 66 Ringer was replaced by the 6th specimen; at 68 by the 4th specimen (collected 3½ minutes after cervical cord section); at 69 by the 2nd specimen (collected before cord section). The bloods 66-69 were diluted with 10 volumes Ringer. Reduced to one-half.



- 10.00 a.m. Started artificial respiration (cat still breathing well spontaneously).  
 10.07 a.m. Intravenous injection of 0.35 mgm. strychnine (sulphate, as in all the experiments).  
 10.07½ a.m. Clonic convulsions; spontaneous breathing good.  
 10.22 a.m. Clonic spasms; spontaneous breathing good.  
 10.23 a.m. Third adrenal specimen, 1.05 gram in 1 minute.  
 10.24 a.m. Fourth adrenal specimen, 3.85 grams in 3 minutes (1.28 gram per minute).  
 10.30 to 10.32 a.m. Cut right and left major and minor splanchnics (in abdomen) and extirpated the left semilunar ganglion.  
 10.34 a.m. Clonic spasms at intervals.  
 10.38 a.m. Fifth adrenal specimen, collected for 30 seconds.  
 10.38½ a.m. Sixth adrenal specimen, 2.7 grams in 10 minutes (0.27 gram per minute).

Another specimen of indifferent (venous) blood was now obtained. Combined weight of adrenals, 0.25 gram. Section of nerves verified at autopsy.

Figures 12 to 15 give samples of the tracings used in the assay in cat 431.

The 2nd adrenal specimen was found to be stronger than 1:8,300,000, weaker than 1:5,000,000, weaker than 1:6,600,000 adrenalin. It was assayed at 1:7,500,000, corresponding to an output of 0.00045 mgm. per minute for the cat, or 0.00023 mgm. per kgm. per minute.

The 4th specimen, obtained after administration of strychnine, was decidedly stronger than 1:1,660,000, stronger than 1:830,000. It was much stronger than the 6th specimen, collected after section of nerves to the adrenals in spite of the greatly diminished flow for the 6th specimen. This was evident when both specimens were diluted with 3 volumes of Ringer's solution, but became still more apparent on first diluting each with 3 volumes of indifferent blood and then diluting the mixtures with 3 volumes of Ringer (fig. 12). The 4th specimen diluted with 3 volumes of indifferent blood was weaker than 1:1,660,000 adrenalin, i.e., the 4th specimen was weaker than 1:415,000 (fig. 13). The 4th specimen was finally assayed at 1:700,000, corresponding to an output of 0.0018 mgm. per minute for the cat, or 0.0009 mgm. per kgm. per minute, four times the initial output.

The 6th specimen, diluted with 1 volume of indifferent blood, was weaker than 1:3,300,000, i.e., the 6th specimen was weaker than 1:1,650,000. Undiluted with indifferent blood it was found to be stronger than 1:3,300,000, but decidedly weaker than 1:1,660,000 (fig. 14). It was assayed at 1:2,000,000, corresponding to an output of 0.00013 mgm. per minute for the cat, or 0.00006 mgm. per kgm. per minute, only one-fifteenth of the output under strychnine before section of the nerves.

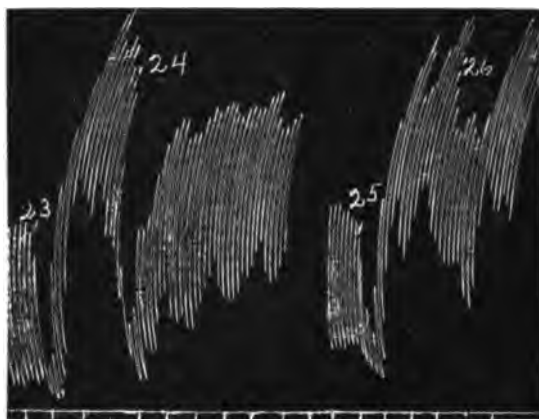


Fig. 12. Intestine tracings. Bloods from cat 431. At 23 and 25 Ringer was replaced by indifferent (venous) blood and this at 24 by the 4th adrenal specimen (collected 17 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 26 by the 6th adrenal specimen (collected after section of nerves to both adrenals) diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer. Reduced to one-half.

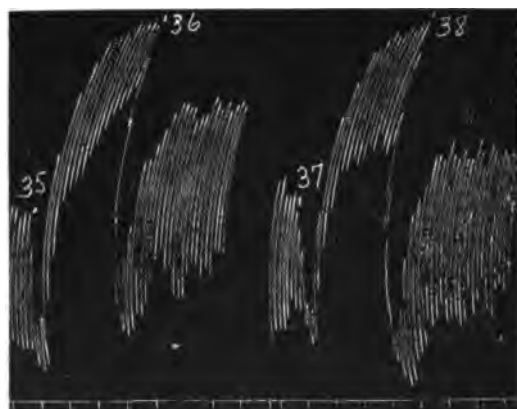


Fig. 13. Intestine tracings. Bloods from cat 431. At 35 and 37 Ringer was replaced by indifferent (venous) blood and this at 36 by the 4th adrenal specimen (collected 17 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:1,660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

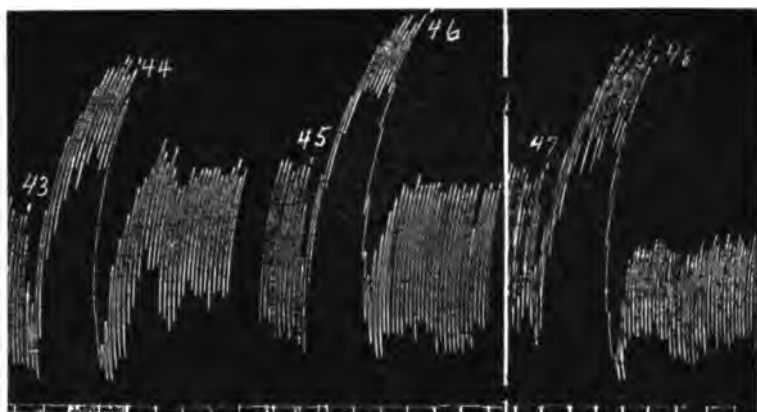


Fig. 14. Intestine tracings. Bloods from cat 431. At 43, 45 and 47 Ringer was replaced by indifferent (venous) blood and this at 44 by indifferent blood to which was added adrenalin to make a concentration of 1:3,300,000; at 46 by the 6th adrenal specimen (collected after section of nerves to both adrenals); at 48 by indifferent blood to which was added adrenalin to make a concentration of 1:1,660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.



Fig. 15. Uterus tracings. Bloods from cat 431. At 51 Ringer was replaced by the 4th adrenal specimen (collected after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 52 by the 6th adrenal specimen (collected after section of nerves to both adrenals) diluted with 1 volume of indifferent blood; at 53 by indifferent (venous) blood; at 54 by the 2nd adrenal specimen (collected before injection of strychnine); at 55 by the 4th adrenal specimen; at 56 by the 4th adrenal specimen diluted with 1 volume of indifferent blood. All the bloods were diluted with 3 volumes Ringer. Reduced to two-thirds.

It was confirmed by the uterus that the 4th specimen, diluted with 3 volumes of indifferent blood, was stronger than the 2nd specimen undiluted with indifferent blood, and at any rate not weaker than the 6th specimen diluted with 1 volume of indifferent blood. The 4th specimen was clearly much stronger than the 2nd (fig. 15).

This experiment is a complete contrast to that on cat 318 in which the cervical cord was transected after the administration of strychnine. There the output of epinephrin, increased by the strychnine, went on unchanged. In the present experiment strychnine markedly increased the output, but even incomplete section of the peripheral adrenal secretory paths caused a great diminution.

#### ACUTE EXPERIMENTS ON OTHER ANIMALS THAN CATS

Table 2 summarizes the results of acute experiments on cervical section in 3 dogs, 2 rhesus monkeys and 1 rabbit. Two experiments on dogs in which the cord was semisected in the cervical region are included in the table. In the dogs and monkeys the output after cervical cord transection was always less than before the transection, except in one of the monkeys where the initial output was abnormally low (shock after exposure of the cord). In this case, as noted in cats under similar conditions, transection of the cord caused no further diminution in the epinephrin output. Semisection of the cervical cord in dogs yielded results of the same general character as in cats. In one of the experiments in the table there was no change in the output after the semisection, in the other it was reduced.

In addition to the acute experiments on dogs summarized in table 2, a number of experiments with blood pressure auto-assays were made. For example, in a dog (351) weighing 6.3 kgm. the output was estimated at 0.00015 mgm. per kgm. per minute. The blood pressure was 94 mm. of mercury. The cord was then transected through the 5th cervical pair. The blood pressure fell to 55 mm. of mercury. The output of epinephrin was now estimated at 0.00003 mgm. per kgm. per minute. At the end of the experiment a sample of adrenal blood, collected at the rate of 1.0 gram per minute assayed (on rabbit segments) at 1:3,000,000 corresponding to an output of 0.00005 mgm. per kgm. per minute.

## SURVIVAL EXPERIMENTS

In table 3 are displayed the results of experiments on 6 cats and 4 dogs which were allowed to survive transection of the cervical cord for 2 to 13 days. It will be seen that while in most of the animals an output of epinephrin measurable by segments of the sensitiveness employed was present, the output never attained the normal average in animals with intact cord. In the dogs the average output after cervical cord transection is considerably greater than in the cats, the opposite of what is found in the acute experiments (see tables 1 and 2). The suggestion is that the mechanism in the thoracic cord is more easily depressed by the cervical transection (spinal shock), but recovers more easily in survival experiments in the dog than in the cat. We do not know whether this apparently easier recovery means anything more than that the dogs in general in our survival experiments did withstand cervical transection better than the cats. They ate better, passed urine and feces more normally and lived longer. Most of the cats during the time they lived or were permitted to live neither micturated nor defecated, although the bladder was generally much distended with urine. In several instances the bladder was artificially emptied in the cats. This was not necessary in the dogs. It is well known that after these high cord transections one of the most important precautions to be taken is to keep up the body temperature. Although all the animals were kept in a room specially warmed day and night, the dogs were somewhat larger than the cats and therefore it is to be supposed would not cool so easily, but we do not know whether this was a factor in their better condition. Most of the animals were in fair, some (including those taken after the longest periods) in very good condition when sacrificed. It would be desirable to make observations on animals after much longer periods of survival.

While it is clear from these experiments, as from the previous ones, that a substantial liberation of epinephrin, sustained from the thoracic cord, may be present in animals which have survived cervical cord transection 2 to 13 days, it is impossible to say whether with a longer survival period the output would approach more nearly to the normal average or not. Great quantitative and possibly some qualitative changes in metabolism follow such a lesion, and in the present state of our knowledge it would be useless to speculate as to how these might affect the upbuilding or output of epinephrin. Mere inanition for 3 days, and all these animals, of course, take little food for some time

TABLE 3  
Acute experiments. Dogs, etc.

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD										REMARKS
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute	For animal	Per kilogram	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute	For animal	Per kilogram		
327	7.4	v-vi	0.615	12.35	30	24.7	130	1:15,000,000	0.0016	0.0002	0.0003	9.5	60	9.5	38	1:40,000,000*	0.00003*				
				10.0	120	5.0	36	1:40,000,000*													
352	6.5	below vi	1.886	9.4	120	4.7	98	1:3,300,000	0.0014	0.0002	0.0006	6.4	120	3.2	72	1:5,000,000	0.0006	40.0001			
380	5.5	v-vi	1.082	9.7	60	9.7	120	1:4,500,000	0.002	0.00035		2.25	240	(a) 0.56	40	1:1,900,000	0.0003	0.000055			
												4.1	120	(b) 2.05	52	1:8,500,000	0.00025	0.000015			
																	</				

After cord section blood pressure fell to 18 mm. of mercury; 300 cc. Ringer injected at once and blood pressure rose to 72 mm. of mercury

Ringer's solution injected between collection of (a) and (b). 100 cc.

328	1.87	vii	0.412	4.5	180	1.5	58	1:9,000,000	0.00017	0.0001	4.45	120	2.22	62	1:13,000,000	0.00017	0.0001	50 cc. Ringer injected before and 50 cc. after cord section
250	8.8	vi	0.796	9.2	150	3.7		1:8,000,000	0.00045	0.00005	10.25	120	5.12		1:13,000,000	0.0004	0.000045	Ringer injected after cord section. 100 cc.
276	8.4	below viii	0.947	6.1	60	6.1		1:5,000,000	0.0012	0.00014	5.05	150	2.0		1:9,000,000	0.00022	0.000026	Assay of specimen, taken after cord section, completed next day
379	4.8	above vi	0.894	8.15	90	5.4	140	1:3,000,000	0.0018	0.00037	7.5	180	2.5	97	1:1,400,000	0.0018	0.00037	Both adrenals discharging
382	7.2	iv-v	0.870	10.95	90	(a) 7.3	120	1:4,000,000	0.0018	0.00025	5.1	120	(b) 2.6	90	1:5,000,000	0.0005	0.00007	(a) before semi-section; (b) after semi-section; (c) after transection below v. Both adrenals discharging
											4.2	180	(c) 1.4	44	1:2,800,000	0.0005	0.00007	

*Note.* 379 and 382 were semisections (right), the rest transections. 328 was a rabbit, 250 and 276 monkeys, the rest dogs.

\* These are the maximum concentrations and output which could have been present. The segment could have detected these concentrations had they been present in the adrenal bloods.

after the operation, does not appear, so far as can be judged from one experiment, to have any notable influence. Thus a cat weighing 2.22 kgm. received no food for 3 days. The stomach and intestines were empty except for some feces in the lower colon. Under urethane specimens of adrenal blood were obtained in the usual way. The 2nd specimen (1.67 gram per minute) was assayed at 1:4,000,000 epinephrin, corresponding to an output of 0.00042 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute. The 4th specimen (1.27 gram per minute) was assayed at 1:2,800,000 epinephrin, corresponding to 0.00045 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute.

It was shown that strychnine exerted its stimulating effect upon the epinephrin output in these survival experiments, doubtless by acting upon the thoracic cord mechanism, which was already sustaining whatever output was going on. Where the initial output is quite small, as in a cat (424) in which the transection was made not in the cervical region but between the first and second dorsal segments, the relative increase produced by strychnine may be very great.

*Condensed protocol. Cat 424; female; weight, 2.1 kgm.*

December 9. Excised left superior cervical ganglion.

December 20. Cord transected between origins of 1st and 2nd dorsal nerves. Immediately after cord section (while still anesthetized), the left pupil became wider than the right (both dilated), but on recovery the left pupil contracted and left nictitating membrane came forward as in animals without cord section.

December 24. Condition good. Left pupil contracted and nictitating membrane forward.

10.10 a.m. Administered a little ether to insert tracheal and jugular cannulae and obtained indifferent (jugular) blood. Isolated right and left vago-sympathetic nerves on loose ligatures. Thereafter no more ether was needed.

10.30 a.m. Left pupil contracted and nictitating membrane forward.

Cut left, then right vago-sympathetic nerves. After section of the right vago-sympathetic both pupils became smaller but the left remained smaller than the right; left nictitating forward.

10.45 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied, in addition to the veins entering the cava pocket. The operative field was of course totally insensitive owing to the cord section.

10.50 a.m. Left pupil wider than right; both nictitating membranes forward.

10.52 a.m. Left pupil wider than right; left nictitating back; left aperture wider than right.



- 10.55 a.m. to 11.20 a.m. Two 2-minute and one 3-minute pocket observations gave no reactions with the pupil; 0.5 cc. 1: 650,000 adrenalin gave a good pupil reaction. Collected adrenal blood.
- 11.30 a.m. First specimen, 0.45 gram in 30 seconds (0.9 gram per minute).
- 11.30½ a.m. Second specimen, 5.05 grams in 6 minutes (0.84 gram per minute).
- 11.42 a.m. Started artificial respiration.
- 11.45 a.m. Intravenous injection of 0.5 mgm. strychnine. In a few seconds a tonic convulsion occurred which was followed after a few seconds by clonic spasms. During the spasms the left pupil became maximally dilated, the right pupil about one half dilated, the left nictitating membrane retracted and the right forward. On clipping off the pocket for 3 minutes the left pupil came down somewhat and the nictitating membrane came forward slightly, but the left pupil remained wider than the right.
- 11.51½ a.m. Third adrenal specimen, 0.5 gram in 30 seconds (1.0 gram per minute).
- 11.52 a.m. Fourth adrenal specimen, 3.7 grams in 7 minutes (0.53 gram per minute). On release of the pocket clip after collecting the 4th specimen, a good pupil and nictitating reaction was observed on the left side although the left pupil was already wide). Another specimen of indifferent blood was now obtained.

The 2nd specimen (taken before strychnine injection) was assayed at 1:150,000,000. This corresponds to an output of only 0.0000055 mgm. per minute for the cat, or 0.0000025 mgm. per kgm. per minute, one-hundredth of the normal average. Figure 16 gives some of the tracings used in the assay of the 2nd specimen. It was weaker than 1:130,000,000 adrenalin, stronger than 1:195,000,000. Other tracings, not reproduced, showed that it was much weaker than 1:65,000,000. Fortunately the segment was exceptionally sensitive, as will be seen from the excellent reactions given with blood so poor in epinephrin. It must be remembered that, as applied to the segment, the bloods being diluted with 3 volumes of Ringer's solution, the actual concentrations of epinephrin were only one-fourth of those given. This illustrates a point of technique on which we have often insisted, but which is still overlooked by some investigators, namely, that the mere occurrence of a large inhibitory reaction of an intestine segment gives no information as to the concentration of epinephrin in the blood producing it until the sensitiveness of the segment for epinephrin has been quantitatively determined.

It was shown that the 4th specimen, collected after strychnine, was much stronger than 1: 6,500,000, and stronger than 1: 1,300,000 adrenalin. Diluted with 3 volumes of indifferent blood it was not much different from 1:3,900,000, i.e., the 4th specimen was not far from

TABLE 3  
Cervical cord sections. Survival experiments

NUM- BER OF ANIMAL	BODY WEIGHT	LEVEL OF CORD SECTION	PERIOD OF SURVIVAL	COMBINED WEIGHT OF ADRENALS	BLOOD COL- LECTED	DURATION OF COLLECTION	BLOOD FLOW PER MINUTE	EPINEPHRIN CONCENTRATION	EPINEPHRIN OUTPUT PER MINUTE		REMARKS
									For animal	Per kilo- gram	
			Days	gms.	gms.	sec.	gms.		mgm.	mgm.	
<i>Cats</i>											
332	2.02	vii	2	0.318	3.95	240	1.0	1:30,000,000*	0.00003*	0.000015*	
336	2.53	vii	3	0.383	4.2	720	0.35	1:3,500,000	0.0001	0.00004	
337	2.6	above vi	3	0.535	3.85	180	1.3	1:30,000,000*	0.00004*	0.000015*	Ether administered between collection of these specimens
					2.75	300	0.55	1:30,000,000*	0.000015*	0.000006*	
346	1.72	vi	7	0.378	4.55	300	0.91	1:25,000,000	0.000036	0.00002	Blood pressure at beginning of experiment was 80 mm. of mercury
					2.35	420	0.34	1:10,000,000	0.000034	0.00002	
420	1.6	below vi	3	0.290	3.35	600	0.335	1:125,000,000	0.0000025	0.0000015	Pupil assay showed output was not more than 0.000025 mgm. per kgm. per minute
422	2.4	below viii	2	0.346	2.55	180	0.85	1:25,000,000	0.000035	0.000015	
419	1.57	below vii	20	0.461	3.95	240	1.0	1:30,000,000	0.000033	0.00002	

<i>Dogs</i>												
330	4.0	vii	2	0.810	4.2	600	0.42	1:10,000,000	0.00004	0.00001	Very small blood flow. Circulation feeble. Temperature low	
345	3.95	vii-viii	8	0.930	8.55	150	3.4	1:12,000,000	0.00028	0.00007	Ether administered between collection of these specimens	
					5.0	180	1.7	1:8,500,000	0.00002	0.00005		
347	2.93	vii-viii	13	1.008	9.7	120	4.8	1:40,000,000	0.00012	0.00004	0.5 mgm. strychnine administered between collection of these specimens	
					5.35	240	1.34	1:6,600,000	0.00002	0.00007		
395	4.5	v-vi	11	0.938	5.8	90	3.9	1:60,000,000	0.000065	0.000015	1st specimen collected without etherisation. Blood pressure 94 mm. of mercury	
					4.25	90	2.8	1:60,000,000	0.000045	0.00001	2nd specimen collected after etherisation. Blood pressure 76 mm. of mercury	
386†	5.25	below v	8	0.831	11.25	120	(a) 5.6	1:8,500,000	0.00066	0.00013	(a) Both adrenals discharging	
					4.85	120	(b) 2.42	1:6,500,000	0.00035	0.000035	(b) Right adrenal only discharging	
					4.85	120	(c) 2.42	1:6,500,000	0.00035	0.000035	(c) Left adrenal only discharging	

\* These are maximum outputs and concentrations which could have been present. The segments, which were not particularly sensitive, could have detected these concentrations had they been present in the adrenal blood.

† 386 is a hemisection experiment (left); the others are transections.

1:1,000,000. It was decidedly stronger than 1:1,625,000 (fig. 17, observations 34 and 36), weaker than 1:650,000 (fig. 17, observations 38 and 40). Another series of observations was made in which the 4th specimen was diluted with 7 volumes and with 14 volumes of indifferent blood, in order to reduce the inhibitory reactions so as to permit of a more exact assay. In this way also it was shown that the specimen was stronger than 1:1,300,000 and weaker than 1:870,000. It was finally taken at 1:1,000,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute, the normal average, and not less than one hundred times the output before strychnine. It was confirmed by the uterus that the

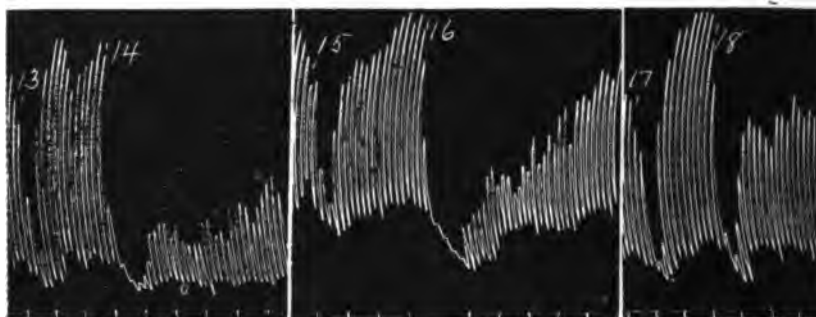


Fig. 16. Intestine tracings. Bloods from cat 424. At 13, 15 and 17 Ringer was replaced by indifferent (venous) blood and this at 14 by indifferent blood to which was added adrenalin to make a concentration of 1:130,000,000; at 16 by the 2nd adrenal specimen (collected before injection of strychnine); at 18 by indifferent blood to which was added adrenalin to make a concentration of 1:195,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

4th specimen gave a good epinephrin reaction, whereas the 2nd specimen and indifferent blood in the same dilution gave no reaction. The uterus was not particularly sensitive.

Advantage was taken of the fact that it was possible to obtain adrenal vein blood without the administration of an anesthetic to test the question whether ether causes any measurable increase in the output after transection of the cervical cord. In no case was any such effect produced. With intact central nervous system we have previously found that the average output is if anything somewhat less with ether than with urethane (6). An observer who trusts to the (denervated)

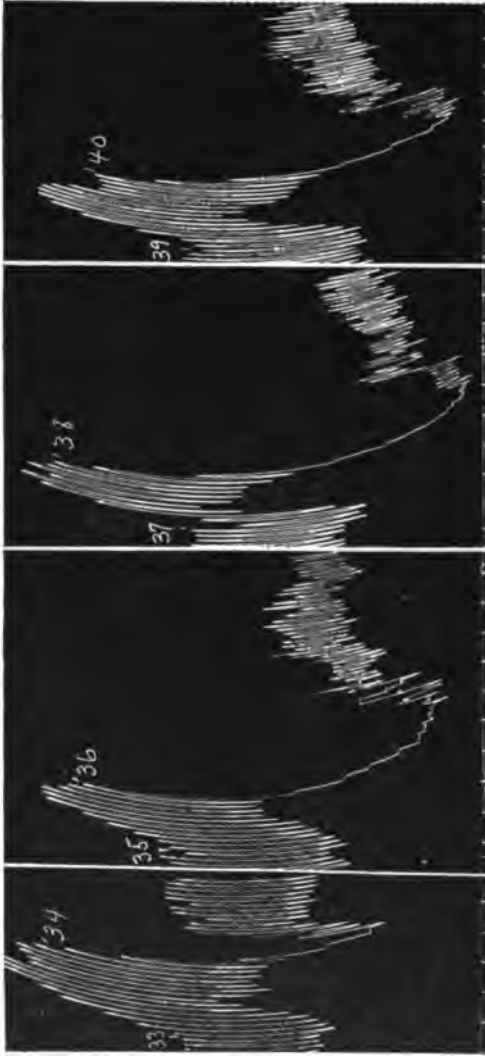


Fig. 17. Intestine tracings. Bloods from cat 424. At 33, 35, 37 and 39 Ringer was replaced by indifferent (arterial) blood and this at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:6,800,000; at 36 by the 4th adrenal specimen (collected 8 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at 40 by the 4th adrenal specimen diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

eye reactions alone can easily be deceived by the fact that ether increases the sensitivity of the test object to epinephrin. For instance (in cat 336), a 2-minute pocket without anesthetic gave no reaction. Ether was then administered and now a 2-minute pocket gave an excellent pupil reaction. This was confirmed several times. It was shown that the increased reaction could not be interpreted as due to an augmentation of the epinephrin output by etherization since the reaction to one and the same dose of adrenalin artificially injected was also increased by giving ether. This is illustrated in the following experiment.

*Condensed protocol. Cat 423; female; weight, 1.8 kgm.*

December 9. Excised left superior cervical ganglion.

December 20. Left pupil contracted and nictitating membrane forward. Transected cord below origin of 8th pair of cervical nerves. Immediately after the cord section the left pupil was wider than the right and the right nictitating protruded more than the left.

December 23. Condition good. Left pupil wider than right as it had been since the cord section, both nictitating membranes slightly forward, apertures of both eyes about the same.

9.45 a.m. Administered a little ether to insert tracheal and jugular cannulae.

On etherizing, the left pupil became almost maximal, the right pupil dilated but not so widely as the left, the right nictitating protruded more than the left.

10.15 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied in addition to the veins entering the cava pocket.

10.16 a.m. Left pupil  $\frac{1}{2}$  dilated; right pupil slit-like; left aperture slightly wider than right.

10.18 a.m. Pocket observation 2 minutes; no eye reaction.

10.22 a.m. Pocket observation 3 minutes; no eye reaction.

10.25 to 10.35 a.m. 0.5 cc. adrenalin 1:2,700,000, and 0.5 cc. adrenalin 1:2,000,000 gave no reaction; 0.5 cc. adrenalin 1:1,330,000 gave a pupil reaction in 11 seconds.

10.35 a.m. Pocket observation 4 minutes, doubtful reaction if any.

10.40 a.m. Etherized to surgical anesthesia.

10.45 a.m. Pocket observation 3 minutes gave a pupil reaction in 14 seconds and retraction of nictitating membrane 2 seconds later.

10.50 a.m. 0.5 cc. adrenalin 1:2,000,000 gave a very good pupil reaction in 11 seconds.

10.55 a.m. 0.5 cc. adrenalin 1:2,700,000 gave a definite reaction in 8.6 seconds; 0.5 cc. adrenalin 1:4,000,000, doubtful if any reaction.

This experiment brings out another point, namely, the reversal of the eye phenomena after section of the cord, which Schafer (7) has called attention to as occurring after section of the cervical sympathetic

on the side opposite to that on which the superior cervical ganglion has been excised. The same reversal was observed on section of the cord just below the 8th cervical segment in cat 422. The left superior cervical ganglion (in cat 422) was excised a fortnight before section of the cord. Up to the time when the cord was divided the left pupil was smaller than the right and the left nictitating membrane was less retracted than the right. On section of the cord the left pupil became wider than the right and the right nictitating protruded further than the left. This condition persisted for the two days during which the animal was allowed to survive. At the end of the experiment in which adrenal blood was obtained, the observation was made when the animal was being bled to death that with the onset of asphyxia the left pupil dilated widely but not so widely as the right which went to maximal dilatation. The right nictitating became completely retracted, while the left was still visible. The right aperture became wider than the left and remained so until death. No anesthetic was administered as of course the whole operative field was completely insensitive. In cat 424, in which the cord was transected between the first and second dorsal segments, the reversal of the eye phenomena was not seen.

#### OBSERVATIONS ON THE ADRENAL EPINEPHRIN STORE AFTER TRANSECTIONS OF THE CORD

In many of the animals the epinephrin store of the adrenals was estimated at the end of the experiment. The results are collected in table 4. In the two acute experiments, one on a monkey, the other on a dog, the load is less than would be expected in a normal animal killed without anesthetic. But these animals were necessarily anesthetized for some time before the cord transection, and thereafter, nothing can be deduced from this as to any influence of the cord lesion on the store. In the survival experiments, in many of which little or no anesthetic was required for the final experiment, the field of operation being insensitive, there is no conspicuous difference in the store from what would be expected in normal animals. If it looks somewhat too low in some of the animals, it is fully as great as the normal load in others. The effect of post-operative depletion, following the initial operation in which the cord was divided cannot be altogether excluded, although it is not evident. For example, in cat 419, which was taken 20 hours after the cord section, there was a full load.

TABLE 4

NUM- BER OF ANIMAL	KIND OF ANIMAL	WEIGHT OF ADRENALS		EPINEPHRIN CONTENT		REMARKS
		Left	Right	Left	Right	
		grams	grams	mgm.	mgm.	
333	Cat	0.231	0.230	0.16	0.16	Cervical cord section below 6th seg- ment: survival 3 days
335	Cat	0.151	0.155	0.16	0.16	Cervical cord section above 7th seg- ment: survival 3 days
336	Cat	0.197	0.186	0.14	0.14	Cervical cord section through 7th seg- ment: survival 3 days
337	Cat	0.257	0.278	0.16	0.17	Cervical cord section above 6th seg- ment: survival 3 days
346	Cat	0.192	0.186	0.23	0.23	Cervical cord section through 6th segment: survival 7 days
348	Cat	0.228	0.224	0.26	0.26	Dorsal cord section below 5th seg- ment: survival 6 days
419	Cat	0.241	0.220	0.26	0.26	Cervical cord section below 7th seg- ment: survival 20 hours
420	Cat	0.152	0.148	0.15	0.15	Cervical cord section below 6th seg- ment: survival 3 days
421	Cat	0.230	0.218	0.22	0.22	Cervical cord section through 7th segment: survival 4 days
422	Cat	0.184	0.162	0.13	0.14	Cervical cord section below 8th seg- ment: survival 2 days
423	Cat	0.208	0.200	0.19	0.19	Cervical cord section below 8th seg- ment: survival 3 days
338	Dog	0.397	0.372	0.42	0.40	Cervical cord section below 7th seg- ment: survival 5 days
345	Dog	0.480	0.450	0.46	0.47	Cervical cord section below 7th seg- ment: survival 8 days
347	Dog	0.488	0.520	0.64	0.66	Cervical cord section below 7th seg- ment: survival 13 days
349	Dog	0.722	0.708	1.31	1.33	Dorsal cord section below 5th seg- ment: survival 7 days
352	Dog	0.964	0.922	0.41	0.42	Acute experiment: morphine and ether: cervical cord section below 6th segment: also dorsal section
394	Dog	0.406	0.352	0.47	0.46	Cervical cord section below 5th seg- ment: survival 8 days
395	Dog	0.520	0.418	0.48	0.48	Cervical cord section below 5th seg- ment: survival 11 days
276	Monkey	0.480	0.467	0.20	0.20	Urethane anesthesia: acute experi- ment: cord section below last cer- vical segment: adrenals assayed next day



TABLE 5

NUMBER OF ANIMAL	WEIGHT OF ADRENALS		EPINEPHRIN CONTENT		REMARKS
	Left	Right	Left	Right	
	grams	grams	mgm.	mgm.	
339	0.212	0.186	0.26	0.20	Under ether cervical cord sectioned below 7th segment, left adrenal excised and ether administered for 3 hours and 15 minutes, then removed right adrenal
340	0.160	0.148	0.16	0.16	Cat died 8 days after denervation of left adrenal and 2 days after cervical cord section between 5th and 6th segments
341	0.330	0.300	0.34	0.34	Left adrenal denervated 9 days; cervical cord transected below 6th segment 3 days; cat dying when adrenals were removed
342	0.344	0.320	0.31	0.31	Left adrenal denervated 9 days; cervical cord transected below 6th segment 3 days; ether 2 hours; cat in moribund condition
343	0.210	0.236	0.25	0.16	Under ether sectioned cervical cord (between 5th and 6th segments); excised left adrenal; then morphine 60 mgm., injected hypodermically; removed right adrenal 5½ hours later
344	0.22	0.24	0.21	0.21	Under ether, sectioned cervical cord (above 6th segment); excised left adrenal; then 6 cc. of 2 per cent solution of $\beta$ -tetrahydronaphthylamine injected hypodermically; right adrenal excised 5 hours later

In table 5 are given the results on 6 cats in which a differential effect on the two adrenals could have been manifested, either because one gland had been previously denervated or because one adrenal was removed early and the other at the end of the experiment. In the one experiment tried, morphine seemed to produce its ordinary differential depletion after cervical transection, qualitatively at any rate. It would be impossible without more experiments to know whether the effect was as great quantitatively as with intact cord. In the one experiment made,  $\beta$ -tetrahydronaphthylamine caused no differential effect. Both with morphine and  $\beta$ -tetra the usual symptoms were elicited, so far as they could be manifested by the portion of the animal innervated from above the cord lesion. In 3 cats, dead or moribund at the time the adrenals were removed, the store was the same in each gland. This has little significance, as the same may be seen with intact

cord, although often in animals dying in the laboratory after section of the nerves of one adrenal a marked difference in the store has been found.

#### SUMMARY

1. Our previous work on the liberation of epinephrin after the transection of the cervical cord at various levels in acute experiments on cats has been confirmed and extended. The output may be unaltered by the transection, or it may be diminished. Evidence is given that when the output is diminished this is due to "spinal shock" of the mechanism in the thoracic cord concerned in sustaining the epinephrin output. When the bulb and brain were eliminated by a bloodless method (ligation of the head arteries) the output remained uniformly undiminished.

2. In dogs the epinephrin output in acute experiments was always diminished by transection of the cervical cord, owing, it is suggested, to the greater susceptibility to spinal shock of the epinephrin secretory mechanism. The two monkeys examined showed in this regard the same behavior as the dogs.

3. In survival experiments, the output never equalled the average ordinary output, although it was often substantial. In these experiments it appeared that in dogs the output approached more nearly to that found in animals with intact nervous system than in cats, the opposite of what was seen in the acute experiments, as if the secretory mechanism in dogs, although more easily depressed by the spinal section, recovered to a greater degree in the relatively short periods for which the animals were kept alive (up to 13 days). It is not known whether the better general condition of the dogs than of the cats after the operation is a factor in this recovery.

4. Strychnine increases markedly the epinephrin output after transection of the cervical cord both in acute and survival experiments. The action is central (on the thoracic cord).

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## THE RELATION OF CATALASE TO HEART ACTIVITY

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The catalase content of body fluids and solid tissues, particularly the relation of catalase to activity and oxidation processes in general, has been the subject of much investigation in recent years, especially by Burge and his coworkers (1). Their results have been striking in many respects although their methods have been criticised by Becht (2) and also by Reimann and Becker (3).

The results which are obtained in attempting to make quantitative determinations of catalase strongly support Becht's statement that no satisfactory method has yet been devised for the determination of catalase in solid tissue; that is, a method giving uniform and accurate measurements of catalase. It is of course impossible to comminute solid tissue, such as heart muscle, to the same degree of separation twice in succession; but such comminutions may be made to approach each other very closely at least, and, provided the determinations are carried out at the same temperature—and this has seemed to be the more decisive factor—fairly accurate and satisfactory results can be secured.

It is also true, as Becht pointed out, that the oxygen release by the catalase of solid tissue acting upon peroxide is not complete in ten minutes (the time period most frequently used as a standard), but it seems proper to assume a quantitative relationship between the amount of catalase present and the amount of gas evolved in a stated period of time; more especially would such a relationship be properly assumed when it is desired to consider catalase in its relation to activity.

The experiments outlined below were begun before the criticisms were offered and previous to the suggestion of more exact methods by Harvey (4); the method used by Burge for the determination of catalase has been followed although the apparatus for the collection of oxygen much more resembled that used by Becht. Since the results

desired were not primarily mere quantitative ones but rather simply a series of measurements which might be properly comparable with each other there would seem to be no valid reason why such comparisons may not be made with the results secured by Burge's method.

The comparative results presented are based upon the amount of gas evolved by 1 gram of finely comminuted ventricular muscle shaken 200 double shakes per minute with 50 cc. of hydrogen peroxide for a period of ten minutes. Proper adjustments for pressure were always made and any results that are compared were always secured at the same temperature, although a uniform temperature has not been maintained for all determinations.

In one of his earlier papers Burge (5) stated that "the amount of catalase in the different muscles of the body varies with the amount of work done by these muscles; those doing the greatest amount of work contain the most catalase, while the muscles doing least work contain least catalase." He stated further that "by increasing or decreasing the external physical work of a muscle the amount of catalase is correspondingly increased or decreased."

Mary Mitchell Moore (6), in presenting evidence for the enzymatic basis of the heart beat, concludes that "the changes in the heart beat of the *Fundulus* embryo at high temperatures are such as would be expected in case the rhythmical contractions of the heart depend upon the velocity of an enzyme reaction." And further that "since the temperature necessary to bring about standstill of the heart varies with the time of exposure that it indicates a temperature coefficient of the destruction of the enzyme."

From such statements by these two investigators it would seem a proper assumption that the amount of catalase in heart muscle would vary with its activity. To test the truth of such an assumption determinations were made of the catalase present in hearts of turtles (*Pseudemys scabra*) under varying conditions.

It was found most convenient to make the determinations in sets of threes; the results presented are therefore based upon the use of one control heart and two hearts under modified conditions. Several such group determinations were averaged and whenever the turtle heart was of sufficient size, and this was usually true, two determinations were made with each heart. To cite a typical example of the method of procedure: three turtles were taken and the carapace of each was trephined, exposing the heart. A small opening into the pericardium permitted the introduction of a thermometer and the reading thus

taken has been called "blood" temperature. The trephined opening permitted the heart beat to be counted while at the same time it made simple the subsequent warming or cooling of the entire turtle without loss of blood or other disturbance of the heart. The opening was of sufficient size to allow the rapid removal of the heart at any time it was desired to make a determination of the catalase content.

The following table shows some of the results secured by determinations of catalase:

	BLOOD TEM- PERATURE	HEART RATE	TREATMENT	LATER TEM- PERATURE	NEW HEART RATE	GAS EVOLVED (AVERAGE)
	°C.			°C.		cc.
A	1	1½	Control			43
	0 to 1	1½	Heart isolated and warmed	28	30 to 40	33
	1	1½	Whole turtle warmed	27	25 to 30	59
B	18	9 to 15	Control			75
	19	16	Heart isolated and warmed	37	70	61
	16	20	Heart isolated and cooled	7	4½	65
C	-2	None; frozen	Turtle slowly warmed 4 hours	14	6	83
	-2	solid	Same; 8 hours	21	21	73

It will be noted that the table has been sub-divided into three parts, A, B and C, representing groups of determinations made under different conditions.

Group A represents turtles that had been kept for several weeks in an ice-covered tank and in which the "blood" temperature was approximately 1°C. and the average heart rate 1½ per minute. The catalase determinations were made at a room temperature of 17°C. Control hearts immediately isolated evolved an average of 43 cc. of oxygen per gram of ventricular muscle in ten minutes. Isolated hearts subsequently warmed until the heart rate was 30 to 40 at a temperature of 28° evolved an average of 33 cc. of gas, a decrease of approximately 25 per cent. Hearts beating 25 to 30 times per minute in situ as the result of warming the entire turtle to 27° showed an increase of about 35 per cent.

In group B the turtles had been kept at room temperature (23°C.) for six hours previous to the determinations with hearts beating at

rates varying from 15 to 20 per minute, one turtle having an arrhythmic rate of 9. Gas evolved by the control hearts averaged 75 cc. in ten minutes; after warming isolated hearts until the heart rate was 70 and heat rigor was imminent at 37°C., the average amount of oxygen was 61 cc., a decrease of 19 per cent, while after cooling the isolated hearts to 7°C., when the heart rate decreased to  $4\frac{1}{2}$ , the average amount of gas evolved was 65 cc. The difference between the warmed and cooled hearts, or hearts beating 70 times per minute and those beating at 4, is in favor of those at the slower rate—but this is probably to be accounted for by the error of method rather than by any true difference in the catalase content. All determinations in this group were made at 23°C.

Group C is in reality not a "group" as but two turtles were used, although two determinations were made from each. However the results were so striking that they have been included in the table. The turtles had been exposed to a temperature of -6°C. for 15 hours and were frozen solid. After trephining in a warm room the thermometer was forced into the body cavity and registered -2°C. One turtle was slowly warmed for four hours and at the end of that period the heart beat was reestablished, the rate being 6, reflexes had reappeared, and the intra-pericardial temperature was 14°C. The amount of oxygen released by one gram of ventricular muscle was 83 cc. in ten minutes, being the average of two determinations made at room temperature of 23°C.

The second turtle was permitted to remain at room temperature for an additional four hours, eight hours total, and at the end of that time the intra-pericardial temperature was 21°C. with a heart rate of 21 beats per minute. The amount of gas evolved averaged 73 cc., both determinations giving less oxygen than either of the determinations from the heart beating less than one-third as rapidly.

#### SUMMARY

Quantitative determinations by Burge's method of catalase in the ventricular muscle of turtle hearts give no evidence that there is any close relationship between the activity of the heart and the amount of catalase present.

Hearts with a rapid rate as the result of warming varied both plus and minus, as compared to controls, in catalase content, the greater number showing less catalase.

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## THE EFFECT OF VITAMINE DEFICIENCY ON VARIOUS SPECIES OF ANIMALS

### I. THE PRODUCTION OF XEROPHTHALMIA IN THE RABBIT<sup>1</sup>

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It was shown by McCollum (1) and by Osborne and Mendel (2) that the lack of the vitamine or unidentified dietary essential known as fat-soluble A causes a condition of the eyes of rats which is classed as a type of xerophthalmia. The eye-lids become extremely swollen, so that they are opened with difficulty or not at all. The cornea also becomes inflamed, and blindness and death result unless the missing vitamine is supplied before the condition has progressed too far. This specific effect of the lack of fat-soluble A has been observed repeatedly in rats, but consideration of the effect of such deficiency on other species has been generally only by analogy. The occurrence of a condition in children which may have been xerophthalmia was reported from Japan by Mori and from Denmark by Bloch (3). The cases in Denmark were young children who had been fed separator-skimmed milk. Both reported that the patients generally recovered after feeding chicken livers, cod liver oil or whole milk. Cases of eye trouble or blindness in suckling pigs have been occasionally reported to the present authors. The possibility that this may be due to a deficiency of fat-soluble A in the ration of the sows emphasizes further the importance of securing definite information on this subject. The experimental production of this condition in the eyes of rats does not prove, however, that the feeding of a ration deficient in fat-soluble A will produce the same symptoms in another species. The behavior of one species under certain conditions may be exceptional, and instances are on record of the danger of generalization from experiments with a

<sup>1</sup> Published with the permission of the Director of the Iowa Agricultural Experiment Station.



single species. An example is the fact that a diet which produces symptoms of scurvy in a guinea pig does not similarly affect a rat. If the guinea pig requires a certain anti-scorbutic substance, the rat apparently needs a smaller amount or does not need it at all (4).

There are apparently no definite experimental data on the comparative need of different species for fat-soluble A, although it is assumed that all animals need a certain minimum amount of this unidentified substance. It seems possible that the growth impulse may be a factor, or that the dietary habits of the species may influence the relative requirement. Since many herbivorous animals subsist largely on materials rich in fat-soluble A, as green leaves, their relative need for that vitamine may be greater than animals whose natural diet consists largely of seeds and roots. It has been demonstrated that the pig is much more economical with the limited supply of calcium at its disposal than is the dairy cow. This suggests a similar varying requirement for the vitamins also, which are now assumed to be essential factors in the nutrition of all animals.

The study of the requirement of several species of animals for fat-soluble A has been begun by the authors. It is evident from the work of Osborne and Mendel (2) and McCollum (1) that the development of xerophthalmia in the rat is a specific symptom of a marked deficiency in fat-soluble A, and we consider at present that the production of such a symptom in another species is an important step toward determining its relative or absolute requirements for that substance. However, our criterion for the completeness of a ration is not its failure to produce such a definite symptom of malnutrition, but its ability to carry the animal through a normal period of growth and at least one satisfactory reproductive period. Until these points are investigated with other species than the rat the importance of the vitamins in the human dietary, or in the ration of farm animals like the pig, can only be assumed. Furthermore, a deficiency of fat-soluble A in some species may produce some other symptom than xerophthalmia.

#### EXPERIMENTAL

At the outset of this work a little difficulty was found in securing consumption of a purified ration by the rabbits. After the first week, however, the feed was entirely consumed each day until evidence of malnutrition appeared, coincident with loss of appetite. The ration used was as follows:

		<i>Salt mixture</i>	
Commercial casein.....	20	NaCl.....	0.45
Dextrin <sup>2</sup> .....	65	MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	1.10
Salt mixture.....	5	NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O.....	0.85
Lactose.....	5	K <sub>2</sub> HPO <sub>4</sub> .....	2.40
Wheat embryo.....	5	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O.....	1.35
		Ca lactate.....	3.00
		FeSO <sub>4</sub> .....	0.25
		KI.....	trace

To this mixture was added alfalfa meal which had been extracted with hot alcohol for three or four days to remove fat-soluble A. Records were kept of the feed consumed each day. The average daily feed per rabbit was 60 grams of the mixture and 20 grams of extracted alfalfa. This was mixed with enough water to moisten it thoroughly. Additional water was allowed.

Three rabbits which averaged about 800 grams in weight were put on this ration, with one litter mate (rabbit 3) as a control on a ration of oats and alfalfa. It might be stated that our controls now receive the purified ration plus butter-fat instead of oats and alfalfa. After a month on the casein-dextrin ration one rabbit, no. 6, was taken out and given a ration of oats, gelatin, dextrin, salts, agar and extracted alfalfa. To date this animal has shown no evidence of xerophthalmia but has remained thrifty and growing. Further data on this animal will be given in a later publication.

Rabbits 4 and 5 remained in a thrifty condition, growing at a normal rate, as compared to the control animal, for a period of sixty-one days. At this point both rabbits began to lose appetite and the eyes became dull and slightly cloudy. A day or two later white films appeared over part of the cornea. At this point butter-fat was mixed with the ration of animal no. 5, but this rabbit refused to eat even fresh green lettuce and became steadily worse. Five days later the eyes were swollen nearly shut and there was considerable whitish exudation. The appearance of the rabbit at this time is shown in figure 1. The next day the animal died, having become entirely blind and having decreased in weight from 2170 grams to 1375 grams in ten days.

Rabbit 4 continued to eat enough to nearly maintain its weight for ten days after the first appearance of an abnormal condition of the eyes.

<sup>2</sup> Starch was moistened with a 0.5-1.0 per cent solution of citric acid and heated in an autoclave at 15 pounds for 1½ hours. The dextrin was broken up while drying, and finally ground.



**Fig. 1.** Rabbit 5, showing xerophthalmia. Weight, 1375 grams.



**Fig. 2.** Rabbit 4, showing eye-lids swollen shut as result of xerophthalmia. Weight, 1430 grams.

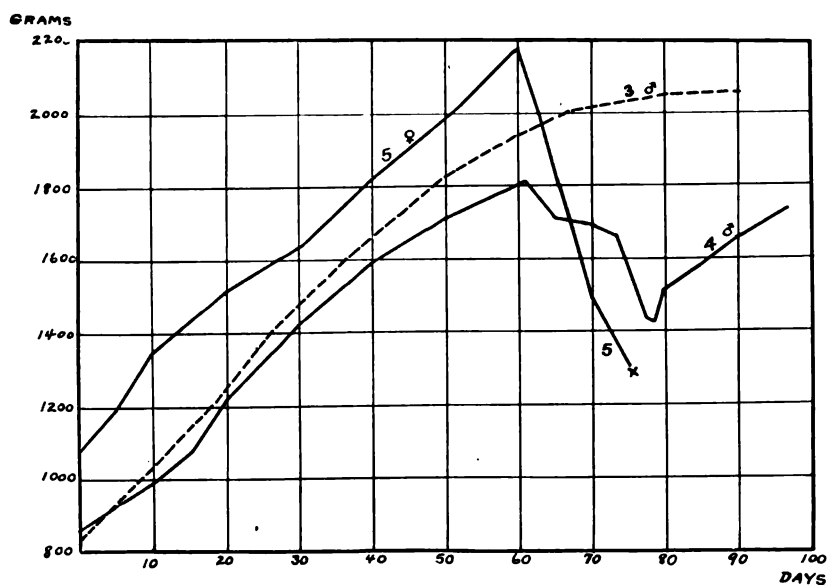


Chart 1. Growth curve of rabbit 3, the control on oats and alfalfa; of no. 5, which died after the onset of xerophthalmia; and of no. 4, which recovered after the administration of butter-fat.



Fig. 3. Rabbit 4, showing recovery of eyes, fifteen days after figure 2. Weight 1652 grams.

At this time, when the eye-lids were swollen nearly shut and were typically congested, the rabbit was photographed (see fig. 2). Ten grams of melted butter-fat were given by mouth with a pipette, and 5 grams each day for four succeeding days were given in the same manner. During these days the regular casein ration was fed and a total of 5 grams of fresh green lettuce and 15 grams of fresh cabbage were given in small amounts to stimulate the appetite. From this point nothing was fed except the regular casein ration to which about 10 per cent butter-fat had been added. In three days after the feeding of butter-fat began the eyes were much improved, and in seven days they were entirely normal in appearance. The animal continued to gain in weight, with increasing appetite and other evidences of well-being, until its loss in weight was entirely recovered (see chart 1). Figure 3 shows the appearance of this rabbit (no. 4) fifteen days after the feeding of butter-fat was begun.

Further experiments on the effect of the lack of fat-soluble A are now in progress, using pigs, guinea pigs, and chickens. We expect to determine the relative requirements of these and other species for this specific vitamine.

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THE FLASHING INTERVAL OF FIREFLIES—ITS TEM-  
PERATURE COEFFICIENT—AN EXPLANATION  
OF SYNCHRONOUS FLASHING

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For several summers past the writers have had opportunity to observe the flashing of Lampyridae. In the summer of 1915 great numbers (species not identified but probably *Photinus pyralis* or *Photurus*) were observed, but only once or twice were the flashings observed to be synchronous. As could be viewed across an open field of about a hundred yards, vast numbers of the insects were flying among the lower branches and at the edge of a wood of oak and chestnut trees. This summer again the phenomenon was observed, but this time quite close at hand in an open lawn, where there could be no possible obstruction to the view. There were a dozen or more beetles flying about three feet above the ground from which they apparently had just risen. The air was perfectly still and very humid. The synchronous character of the flashings continued for six or eight flashes when the insects were lost among some bushes and low trees toward which they had been drifting. The observers were thoroughly convinced then that fireflies, as others before had observed, may at times flash synchronously; and in an effort to gain some light on the cause thereof, the following study was made.

It was first decided to time accurately the interval between flashes of the insects in their normal mating flights. It was soon found that a stop-watch enabling one to read an interval as small as a tenth of a second was a satisfactory instrument. It was soon evident that the temperature was an important factor, and so a good thermometer was hung out in the garden. This practically completed our instrumentarium.

The normal mating flight was chosen for timing the interval between flashes because it is only during this activity that there seems to be

any regularity in the phenomenon. It must have been the experience of others, as it has been ours, that the firefly in captivity will not show any marked regularity in its flashing interval. On the other hand, those individuals that arise in the evening from their day-time haunts and begin to fly about in the air unmolested exhibit a remarkable regularity in the interval between flashes. The deviations in this interval that did occur under such conditions, it seemed to us, were of such an order that they could be easily accounted for by the difference of temperature *a*, of various strata of the air above the earth especially during a calm; *b*, of the bodies of the insects themselves. Those just beginning to fly, and still close to the earth, are probably cooler than those that have been in flight longer. The fact that bees keep themselves warmer in the hive by the activity of their wing muscles would support this assumption. Direct observation of individuals seemed to show some small but constant differences in the flashing interval of insects near their day-time retreats (places moist and cooler than the air itself) and of those higher up in the air, that had been presumably in flight a longer time. The flashing interval of the latter insects was shorter than that of the former.

The duration of the mating flights of the fireflies seemed to be from forty-five minutes to an hour, or about the period of the twilight.

The results of the systematic observations are gathered up in table 1. The individual readings are not recorded but the number of such readings made is stated, their extremes and then their average given together with the temperature of the air. This latter is a mean reading for the evening in each case. For the ten evenings recorded the variations of temperature were respectively 0.4, 0.0, 0.2, 0.75, 0.3, 0.0, 0.2, 1.0, 0.4 and 0.8°C.

The method of making a reading was to record the interval of time between two successive flashes of one individual. It was found impracticable and unnecessary always to follow one and the same beetle for more than one reading. It happened that this could be done sometimes for three or four successive readings and then the interval timed turned out to be the same to the tenth of a second. The intervals for different individuals did not vary greatly for the most part from each other for any one evening, although the extremes, as the table shows, indicate a deviation from 4 to 18 per cent from the mean.<sup>1</sup> In

<sup>1</sup> On the other hand our observations agree with the flashing interval as observed by Mast (1a) for *photinus pyralis* in western Maryland. Curiously, and to our great regret, until now as our own paper is passing through the press we

all the cases where the readings were numerous the average is nearly the same as the mean, so it was thought admissible to incorporate observations for those evenings when the readings were few. For the first two evenings mentioned in the table only the extremes of the observations were recorded, and although the individual observations were numerous, here we have only the mean which is set down in brackets among the averages. Assuming the interval between two successive flashes to be a measure of the rate, this latter was calculated for the minute and set down in the last column of the table. As will be noted this varies from 8 to nearly 16 flashes per minute

TABLE 1  
Summary of observations on the normal flashing interval of fireflies at various temperatures

DATE 1919	TEMPERATURE OF AIR	NUMBER OF OBSERVATIONS	FLASHING INTERVAL IN SECONDS		RATE OF FLASHINGS PER MINUTE ( $\bar{x}$ )
			Extremes	Average interval	
	°C.				
July 25	ca. 28.3	Many	3.5-4.5	[4.0]	15.0
July 26	28.8	Many	3.7-4.1	[3.9]	15.4
July 28	26.0	27	4.2-5.6	4.76	12.6
July 29	22.62	29	5.2-7.0	5.96	10.0
July 31	22.3	30	5.0-7.3	6.03	9.9
August 1	23.2	28	4.9-6.0	5.38	11.1
August 23	24.1	20	4.8-6.0	5.19	11.5
August 24	26.5	11	4.8-5.2	4.93	12.1
August 25	19.4	9	6.5-8.0	7.37	8.1
August 26	17.2	0			

according to the temperature, a rate quite different from that recorded for *Luciola italica*, 80 to 100 by Peters (1), and 60 to 80 by Verworn (2).

It will be noted that as the season advances the number of readings becomes smaller. This is because the insects become fewer. On August 26 not a single firefly was found although we watched for them

have been ignorant of the existence of this excellent article. It should be consulted for its detailed description of the behavior of mating fire-flies.

From Dr. Mast's description it appears that we have probably recorded only the flashing interval of males, since we did not undertake to capture our specimens and identify their sex. The females do not appear to fly during the twilight activity. But since the females flash in reply to the flashing signal only of the male (Mast), one would not anticipate that their interval is greatly different from that of the male.



even longer than usual. The temperature was also the lowest of any of the evenings and probably contributed toward the final disappearance of the firefly for the season.



FIG. 1

In the accompanying figure flashing rate is plotted against temperature in which it is seen that the temperature coefficient for a difference of  $10^{\circ}$ ,  $Q_{10}$ , is nearly 2, and that the curve is convex to the temperature axis.

In table 2 the flashing rates observed near temperatures  $28^{\circ}$ ,  $26^{\circ}$ ,  $22^{\circ}$  and  $19^{\circ}$  respectively are combined and the value of  $Q_{10}$  determined from the formula (3)

$$Q_{10} = \left( \frac{k_1}{k} \right)^{\frac{10}{t_1 - t}} \quad (1)$$

from which it appears that the  $Q_{10}$  value lies somewhere between 1.99 and 1.95. Let us put it at 1.976 and use this value as a basis for the calculation of flashing rates at the temperatures given in the table for comparison with the observed rates. The magnitude of the temperature coefficient already suggests that the flashing reflex is principally dependent upon chemical reactions. This makes it probable that the flashing rate can be calculated from the van't Hoff formula

$$\log_{10} k = a + bt \quad (2)$$

where  $k$  is the observed velocity (in this case the flashing rate) at a given temperature,  $t$ , and  $a$  and  $b$  are constants. These latter values may be easily determined, for the above value of  $Q_{10}$  is itself the quotient of two rates at 10 degrees apart and hence

$$Q_{10} = 10^{10 \cdot b} \quad (3)$$

Since from (1)  $Q_{10} = 1.976$ , from (3)  $10^{10 \cdot b} = 1.976$ , whence  $b = .0295$ . Substituting known values now in equation (2) we find  $a = 1.3314$ . This enables us to use (2) as often as we like for unknown values of  $\log_{10} k$ . Such a procedure carried out for the temperatures given in table 2 shows an agreement between the observed and calculated values as complete as one could desire.

TABLE 2

$t$	$k$ (OBSERVED)	$Q_{10} = 10^{10 \cdot b}$	$k$ (Calculated)
28.55	15.2	$\left. \begin{array}{l} 1.99 \\ 1.95 \end{array} \right\} 1.99$	15.3
26.25	12.35		12.76
22.46	9.99		10.0
19.4	8.1		8.0

From this we conclude that the interval between the normal mating flashes of the fireflies in our garden<sup>2</sup> (other variables remaining fairly constant) is a function of the temperature; that the interval indicates a rate of flashings varying from 8 to 16 per minute between 19 and 29°C.; and that this variation indicates that, whatever else its nature may be, chemical reactions are the processes fundamental to the flashing mechanism.

The physiologist, however, has already classed the flashing of the Lampyridae among the nervous reflexes of animals. It is known to be independent of the phenomenon of luminescence, a process that is practically continuous or at least pitched at a vastly greater rate. But like the respiratory movements or the heart beat the luminescence is at intervals greatly intensified and this we call the "lightning" or "flashing" of the insects. The sudden irregularities or complete cessation of the flashings observed when the insects are disturbed or put in captivity or when they are at rest or feeding is no more reason to

<sup>2</sup>In view of Dr. Mast's statement (1a) this conclusion can only be made for male specimens for the present. See foot note, page 538.

class the phenomenon as arrhythmic than it would be to class thus the flying movements of the bee because they cease when it alights upon a blossom.

Histological studies (4) and histological studies combined with physiological experiments (5) have shown that although not connected directly with the respiratory muscles, as was formerly surmised, the flashings of Lampyridae are nevertheless primarily controlled by "nerves in direct connection with photogenic tissue . . . ." (5). Furthermore, "a direct control relation exists between the photogenic organs and the nerve centers of the head," and most probably there is a direct connection between the optic organs and these centers (6).

That the rate of flashings can not be increased by increasing the rate of an artificial stimulus as has been lately reported by Gates (7), will be easily understood in the light of these remarks and of the results of this study. When the beetles begin their mating flight the flashing mechanism discharges as fast as it can, that is as fast as the temperature permits; and as regularly as it can, that is as regularly as the incident-inhibiting influences permit. If the inhibitions drop to zero the discharges take on a regularity comparable to any other of the well-known rhythmical nervous discharges, such as the respiratory discharge in the medulla of vertebrates, or the discharge in the cord for any of the progression reflexes. But all of these reflexes that have been investigated for the purpose show that they too have a temperature coefficient of the order of chemical reaction velocities (8).

The flashing mechanism of the firefly thus appears not unlike the mechanism of known nerve centers, rhythmically active at times, at others in complete quiescence.

From all this it then appears further that the synchronous flashing of the Lampyridae no longer belongs to the purely accidental nor even necessarily to the extremely rare phenomena of nature. If the extreme deviation from the mean flashing interval is no more than 4 per cent, as it was on one evening of our observations, or even as much as 10 per cent, as it was on another evening (see the data for August 1 and 24 in table 1) it still would be possible for the flashings apparently to occur in unison, that is to occur during the last second, say of an interval of six seconds duration. The necessary conditions for this would include presumably uniformity of moisture, light and air currents as well as temperature.

On the other hand, as far as the individual beetle is concerned, the synchronous flashing would be purely accidental and utterly devoid of

any relation to a higher intelligence or to instinct or any community activity such as one designed to increase the candle-power of a community lighting plant.

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## EFFECT OF DIMINISHED OXYGEN UPON RATE OF NERVE CONDUCTION IN CASSIOPEA

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If an annulus of subumbrella tissue of a scyphomedusa such as *Cassiopea zamachana* be stimulated by an induction shock, a contraction wave may be generated which will proceed in one direction through the ring of tissue and continues to course through it at a uniform rate provided the temperature, salinity,  $\text{CO}_2$  and other factors of the sea-water remain constant.

Harvey (1) observed that such a contraction wave travelled through a ring of *Cassiopea* at the rate of about 77.5 cm. per second for 11 days. Later Mayor (2) found from a study of 8 rings that the contraction wave moved at an average rate of 44 cm. per second at  $28.9^\circ\text{C}$ .; the range among the 8 medusae being from 38.4 to 48.1 cm. per second; and many other unpublished observations made since that time show that about 40 cm. per second is the usual rate in *Cassiopeas* of large size, although each individual has its own characteristic rate, which may vary with age. It will be recalled that Romanes (3) found that the contraction stimulus in *Aurelia* moves at the rate of 22.9 cm. per second.

The question arose as to whether this pulsation was neurogenic, or myogenic or a combination of the two, or whether it was transmitted by the epithelium as well as by the underlying nerve net of the subumbrella of the medusa.

Mayer (4) showed that the stimulus which produced this muscular contraction could pass through recently regenerated tissue which had no muscles and could not contract. Also if a ring of subumbrella tissue be partially immersed in  $\frac{1}{2}$  molecular  $\text{MgSO}_4$  the immersed part soon loses all visible contractibility, yet the stimulus still passes through this non-contracting area and stimulates the normal muscles into contraction. Heating a part of the ring to about  $37^\circ\text{C}$ . will produce the same effect, the muscles of the heated area becoming incapable of contrac-

tion and yet the stimulus which has passed through such heated tissue causes contraction in muscles which have been kept at a normal temperature of about 30°C.

Moreover, if the epithelial and nervous elements be peeled off leaving the underlying muscles intact no contraction stimulus can be transmitted through the muscles which are not overlaid by nerves and epithelium.

Bethe (5) shows that the pulsation stimulus in *Rhizostoma* and *Cotylorhiza* readily passes over parts of the subumbrella where no muscles exist. In 1919 at Tortugas, Florida, I scraped areas such as A, B, C

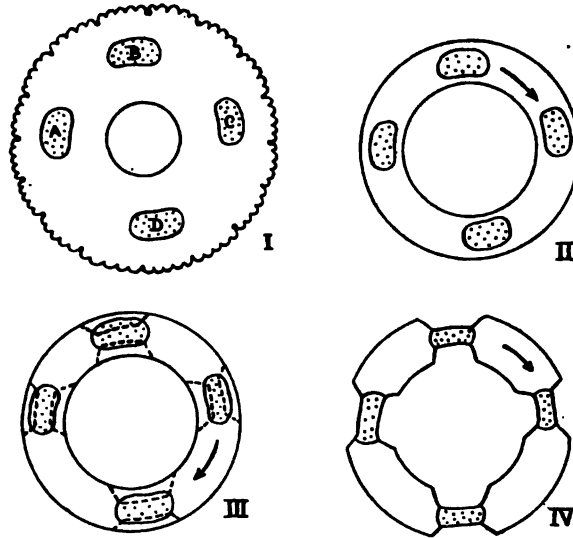


FIG. 1

and D, figure 1, (I), so as to remove all muscular, nervous and epithelial structures. Previous experiments had shown that the epithelium and the nerves regenerate before the muscles. Thus at the end of one or two days the scraped area may be healed over by a thin layer of epithelium underlaid by neurons, but the muscles will not usually develop in less than four or five days. If at the end of a day or two we cut an annulus of subumbrella tissue as in figure 1, (II) and activate it with a contraction wave traveling as shown in the arrow we may then make cuts as shown in figure 1, (III), and transform the ring into the shape shown in figure 1, (IV) in which the contraction stimulus

is forced to pass over areas which lack muscles but have nerves and epithelia. In most of the experiments the stimulus could not be made to pass over all of these regenerated areas for one must usually wait some time *after* the epithelium has healed over before the area can transmit the contraction stimulus. This suggests that the stimulus is transmitted by the nerve net and *not* by the epithelium.

In some cases, however, the stimulus was able to pass over the non-contracting regenerated areas and it was thus possible knowing the length of the regenerated areas in comparison with the circumference of the ring to determine the rate of conduction in the regenerated areas by comparing the rate shown when the ring was in the condition illustrated in figure 1, (II) with the rate shown by figure 1, (IV). In various experiments the rate through the regenerating tissue ranged from 11.5 to 46.5 cm. per second. In the case where the regenerating tissue gave a rate of 11.5 cm. the normal tissue of the same ring had a rate of 39.8 cm. per second; but in the example wherein the rate was 46.5 cm. per second in the regenerating tissue, it was 44.6 in the normal tissue of the same ring, the temperature being 30.2°C. Thus the rate of conduction in the regenerating tissue was from about one-third normal, to fully normal in different cases. During the experiment the regenerating tissue was observed under the microscope to see whether it could contract and after the experiment it was killed in Bouin's fluid stained in Ehrlich's haematoxylin and examined. No muscular elements were detected in the regenerating areas in the experiments reported above. Using Hertwig's osmic acid maceration method, Prof. L. R. Cary made preparations of the subumbrella tissue of *Cassiopea*, and these showed bipolar ganglion cells with their fibers forming a loose net-work underlying the epithelium. One could readily imagine that in young regenerating tissue these fibers would be so few in number that the neuron path might not be completed through the tissue in which case no stimulus could be passed through it, or the path might be less direct than when regeneration had gone farther, in which case the apparent rate across the bridge of regenerating tissue would be slower than when more neurons had developed so as to shorten the zig-zags of the path.

Parker (6) stimulated the pedal region of an actinian (*Metridium*), and found that after an interval the oral disc is retracted. By partly cutting off the pedal edge and leaving it as an attached tongue-shaped strip of tissue, and then stimulating the extreme end and afterwards the basal part of the tongue, he found that the rate of the stimulus through the tongue ranged from 12.1 to 14.6 cm. per second at 21°C.

He claims that this experiment is "the only direct determination of the rate of transmission in the nerve net of coelenterates" but his experiment is, I think, open to the objection that he may be dealing with epithelial transmission, or with a combination of nerve-net and epithelial effects, or with tissue poorly provided with neurons thus giving the very slow rate he observed due to a zig-zag path through the neurons. The studies of Wolff (7) and Havet (8) show that the nervous system is poorly developed in the pedal region of Actinians, and as Parker states the pedal region is incapable of contraction.

Parker raises the objection that the rate of muscular contraction in activated rings of *Cassiopea* may not accord with the rate of the stimulus which passes through the neural or epithelial elements. I find, however, (9) that the muscles of the subumbrella rings are capable of

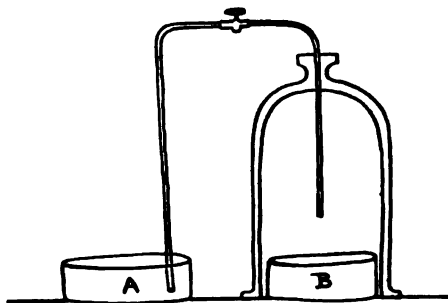


FIG. 2

responding by contractions to stimuli succeeding each other at shorter time intervals than they are called upon to respond to in nature. In other words, in nature they can readily contract to each and every successive recurrence of the stimulus.

It seems certain that in *Cassiopea* the stimulus to which the muscles respond by contraction is neurogenic or epithelial in nature, and the balance of evidence supports the view that it is a neural rather than an epithelial function and we have therefore ventured to call it "nerve conduction."

In 1919 a study was made at Tortugas, Florida, of the relation between the rate of nerve conduction in activated rings of *Cassiopea* and the concentration of oxygen in the sea-water, the oxygen being determined by Winkler's method.

Tortugas sea-water from the surface contains about 4.6 cc. of oxygen per liter of water at 30°C., the oxygen being estimated as at 0°C.



and 760 mm. pressure of mercury. It is thus nearly saturated with oxygen.

The apparatus is shown in figure 2. The activated ring was placed in natural sea-water and its rate determined by counting the number of times per minute the contraction wave traveled around the ring.

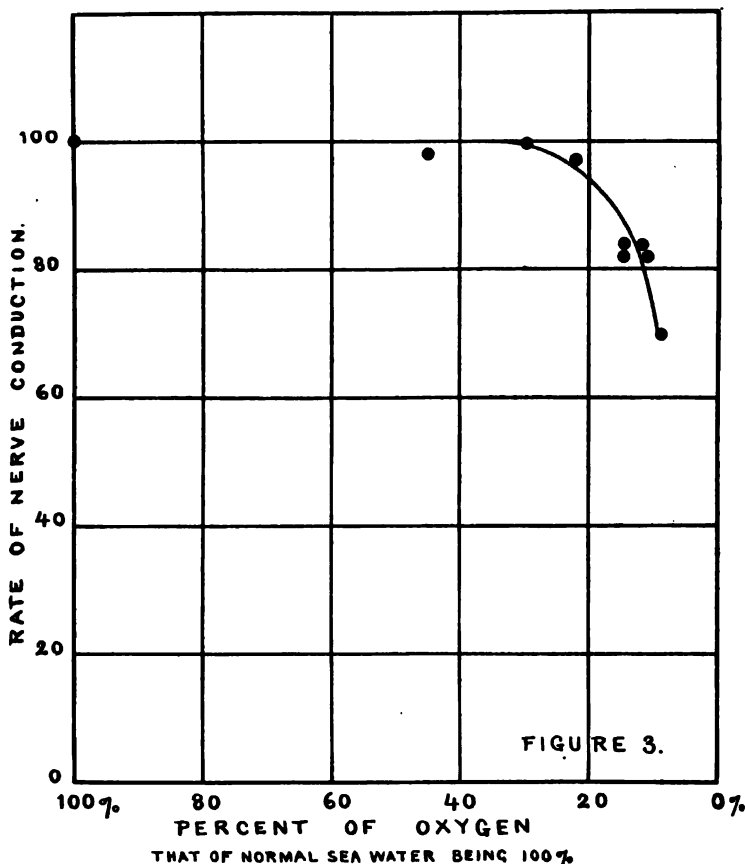


FIG. 3

The temperature of this sea-water was then observed and its oxygen content determined. The pulsating ring was then placed in the glass vessel, *B*, figure 2, under the receiver of an air pump and air was partially exhausted after which sea-water from vessel *A*, was admitted through a fine glass tube. In this manner it was possible quickly to

extract about 96 per cent of the oxygen from the sea-water. When the temperature of the partially exhausted sea-water in vessel *B* had risen to the same temperature as that of the normal sea-water in which the rate had previously been determined, its rate was again ascertained. It was found that after being a few minutes in the partially exhausted sea-water the ring assumed a constant rate which was determined by the degree of concentration of the oxygen in the sea-water. The oxygen in the sea-water in vessel *B* was then determined and compared with that in the natural sea-water in which the rate of the ring had previously been ascertained. These rings appeared to suffer no permanent injury from this treatment for within three minutes after being returned to natural sea-water they were pulsating at normal rate and amplitude.

The results are stated in table 1, illustrated by figure 3.

TABLE 1

*Relative rates of nerve conduction in rings of subumbrella tissue of Cassiopea in normal sea-water and in sea-water with gases reduced by placing it under an air pump. The reduction of gases was estimated by determining the oxygen by Winkler's method.*

CONDITIONS OF THE EXPERIMENT: RATE OF NERVE CONDUCTION IN NATURAL SEA-WATER CONTAINING ABOUT 4.6 CC. OF OXYGEN PER LITER. (THE OXYGEN IS ESTIMATED AS BEING UNDER 760 MM. PRESSURE, AND AT 0°C.)		RELATIVE RATE OF NERVE CONDUCTION
Rate in natural sea-water.....		100
Rate in sea-water having 45 per cent of the normal oxygen supply..		98
Rate in sea-water having 30 per cent of the normal oxygen supply..		100
Rate in sea-water having 21.9 per cent of the normal oxygen supply..		97
Rate in sea-water having 14.3 per cent of the normal oxygen supply..		84
Rate in sea-water having 14.3 per cent of the normal oxygen supply..		82
Rate in sea-water having 11.9 per cent of the normal oxygen supply..		84
Rate in sea-water having 11.5 per cent of the normal oxygen supply..		82
Rate in sea-water having 9.3 per cent of the normal oxygen supply..		70

The curve derived from these experiments is shown in figure 3.

It seems that the rate of nerve conduction and also the amplitude of the muscular contraction remains practically normal until the oxygen of the sea-water is reduced to about 22 per cent of its normal concentration; but further reduction in the oxygen causes a sharp decline both in rate of nerve conduction and amplitude of the contraction. Thus the reaction accompanying nerve conduction seems to require a concentration of only 1 cc. of oxygen per liter, whereas the concentration of oxygen in normal sea-water is about 4.6 cc. per liter.

Nerve conduction in subumbrella rings must usually maintain at least half its normal rate in order to sustain the ring in pulsation, but the normal *Cassiopea* medusa with marginal sense-organs intact can still pulsate in sea-water containing 4 per cent or even less of the concentration of oxygen found in natural sea-water. Thus the rhopalia can still engender the pulsation stimulus and the nerve net can conduct it in a concentration of less than 0.18 cc. of oxygen per liter of sea-water.

That nerve conduction is not a simple process of oxidation is shown by the studies of A. V. Hill (10) who demonstrated that there is no appreciable temperature change during the transmission of the nerve impulse. Also Moore (11) shows that there is no increase in the rate of CO<sub>2</sub> production in nerve-muscle preparations of the frog when the nerves are stimulated by a tetanizing current.

However, we know that nerve conduction cannot be wholly independent of oxygen for von Baeyer (12) showed that hydrogen or nitrogen rendered nerves insensible to induction shock, but this sensibility may be restored by oxygen; and moreover Fröhlich (13) showed that the refractory period is prolonged in absence of oxygen.

#### SUMMARY

In the scyphomedusa *Cassiopea* the pulsation-stimulus is neurogenic.

Nerve conduction remains normal in rate and intensity until the oxygen in the sea-water is reduced to 22 per cent of its normal concentration; thus until there is about 1 cc. of oxygen per 1000 cc. of sea-water, the oxygen being estimated as at 0°C. and 760 mm. pressure of mercury. Further reduction in the concentration of oxygen results in a marked decline in both rate and amplitude of pulsation.

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# THE ALKALI RESERVE OF THE BLOOD PLASMA, SPINAL FLUID AND LYMPH

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## INTRODUCTION

It has been stated by Van Slyke (1) that the alkali reserve of the blood plasma as measured by its carbon dioxide combining capacity is an index of the alkali reserve of the body fluids. As both lymph and spinal fluid are more or less readily obtained from experimental animals, an investigation to determine primarily the alkali reserve of samples of blood plasma, lymph and spinal fluid taken concurrently, was undertaken. It was early seen that in the case of dogs the carbon dioxide combining power of the spinal fluid varied less in any one animal than that of the blood or thoracic duct lymph, and the investigation was therefore extended with the view of studying changes in the alkali reserve of the spinal fluid, following induced variations in the alkali reserve of the blood.

## METHODS

The alkali reserve of blood plasma, lymph and spinal fluid was determined by the method of Van Slyke and Cullen (1).

The blood was aspirated from an artery or vein into a glass syringe containing a trace of powdered potassium oxalate. The oxalated blood was centrifuged within a few moments after withdrawal, and the plasma pipetted off. Lymph was obtained from a cannula placed in the thoracic duct. It was oxalated as collected, to prevent clotting. The spinal fluid was taken as a rule by occipito-atlantoid puncture but occasionally a lumbar puncture was made. The analyses were made within a few minutes after sampling in most instances, but when a delay was necessary the samples were kept in paraffined tubes in an ice chest until the analyses could be made.

In the preliminary experiments venous blood and spinal fluid were taken from dogs while under the influence of morphine. Arterial and venous blood and spinal fluid were taken in several instances from dogs under light ether anesthesia. One series of animals in this latter group received a subcutaneous injection of morphine prior to etherization, while others received ether only. Samples were also taken from dogs under chloroform and alcohol anesthesia, as the protocols indicate.

It is a well-established fact that anesthesia (2) and trauma (3) tend to lower the alkali reserve of the blood.

A number of experiments were performed to determine the effect of prolonged anesthesia and trauma on the alkali reserve of the blood plasma and spinal fluid. Samples of blood and spinal fluid were taken concurrently in these experiments over a period of some hours.

Variations induced in the alkali reserve of the blood plasma and the spinal fluid, as a result of intravenous injections of 5 per cent sodium bicarbonate in distilled water, were followed in another series of experiments. The animals used in this latter series of experiments were in nearly every instance under morphine-ether anesthesia. The samples of blood and spinal fluid were taken concurrently. The readings obtained by the Van Slyke apparatus were reduced to cubic centimeters of  $\text{CO}_2$  at  $0^\circ\text{C}$ . and 760 mm. bound by 100 cc. of blood plasma, lymph or spinal fluid, at the temperature of  $20^\circ\text{C}$ .

We were able to obtain concurrent specimens of spinal fluid and venous blood from a number of human subjects due to the kindness of Doctors Greene and Munroe.

#### RESULTS

<i>Dog 1. 16 kilos. ♂</i>		<i>Alkali Reserve</i>
4:30 p.m.	Morphine gr. 2; atropine gr. $\frac{1}{10}$ subcutaneous	
6:30 p.m.	Blood aspirated from leg vein . . . . .	59
6:40 p.m.	Spinal fluid occipito-atlantoid puncture . . . . .	62
<i>Dog 2. 14 kilos. ♀</i>		
11:45 a.m.	Morphine gr. 1; atropine gr. $\frac{1}{10}$	
12:00 m.	Spinal fluid lumbar puncture . . . . .	61
12:10 p.m.	Blood aspirated from femoral vein . . . . .	52
<i>Dog 3. 8 kilos. ♀</i>		
4:30 p.m.	Morphine gr. 2; atropine gr. $\frac{1}{10}$	
5:20 p.m.	Chloroform and ether inhalation	
5:30 p.m.	Blood aspirated from leg vein . . . . .	36
5:35 p.m.	Spinal fluid by occipito-atlantoid puncture . . . . .	63

*Dog 4. 10 kilos. ♀*

9:30 a.m.	Ether anesthesia	
10:00 to 1:00 p.m.	Class demonstration	
1:00 p.m.	Blood from femoral vein . . . . .	27
1:00 p.m.	Spinal fluid by occipito-atlantoid puncture . . . . .	41

*Dog 5. 18 kilos. ♂*

10:45 a.m.	Under light ether anesthesia	
11:00 a.m.	Spinal fluid by occipito-atlantoid puncture . . . . .	54
11:05 a.m.	Blood from femoral vein . . . . .	49

*Dog 6. 14 kilos. ♂*

10:15 a.m.	Morphine gr. 3; atropine gr. $\frac{1}{16}$ subcutaneous	
10:40 a.m.	Ether inhalation	
10:50 a.m.	Spinal fluid by occipito-atlantoid puncture . . . . .	60
11:00 a.m.	Blood from femoral vein . . . . .	62
11:05 a.m.	Blood from femoral artery . . . . .	58

*Dog 6 (five days later)*

10:50 a.m.	Morphine gr. 2; atropine gr. $\frac{1}{8}$ subcutaneous	
11:30 a.m.	Ether	
11:35 a.m.	Spinal fluid occipito-atlantoid puncture . . . . .	58
11:40 a.m.	Blood from the femoral artery . . . . .	51

*Dog 6 (seven days later)*

10:25 a.m.	Under ether	
10:30 a.m.	Spinal fluid occipito-atlantoid puncture . . . . .	52
10:45 a.m.	Blood from femoral vein . . . . .	44

*Dog 7. 15 kilos. ♂*

5:30 p.m.	Morphine gr. 2 subcutaneous	
5:45 p.m.	Ether and chloroform inhalation	
5:50 p.m.	Spinal fluid, occipito-atlantoid puncture . . . . .	59
5:55 p.m.	Blood from leg vein . . . . .	55

*Dog 8. 12 kilos. ♂*

11:00 a.m.	Ether anesthesia	
11:30 a.m.	Spinal fluid, occipito-atlantoid puncture . . . . .	68
11:35 a.m.	Blood from femoral artery . . . . .	58

*Dog 9. 8 kilos. ♂*

10:00 a.m.	Morphine gr. 2; atropine gr. $\frac{1}{16}$	
11:45 a.m.	Ether anesthesia	
11:50 a.m.	Blood from left femoral vein . . . . .	61
12:00 m.	Spinal fluid, occipito-atlantoid puncture . . . . .	66
12:30 p.m.	Tracheotomy	

12:25 p.m.	Intestinal massage, and intermittent stimulation of sciatic started	
1:30 p.m.	Blood from the external jugular	50
1:32 p.m.	Blood from femoral artery	46
1:35 p.m.	Spinal fluid	58
2:45 p.m.	Respiratory and cardiac failure, thoracic opened and blood taken from left ventricle	19
2:47 p.m.	Spinal fluid	49

*Dog 10. 8 kilos. ♂*

8:00 a.m.	Fed pint of milk	
10:20 a.m.	Ether anesthesia	
10:25 a.m.	Spinal fluid	57
10:30 a.m.	Blood from femoral vein	54
10:40 a.m.	Tracheotomy	
11:15 a.m.	Thoracic duct cannularized	
11:25 a.m.	Pancreatic duct cannularized	
12:00 m.	Lymph collected	43
12:15 p.m.	Blood from femoral vein	40
12:10 p.m.	Spinal fluid	53
12:25 p.m.	Lymph	38
12:30 p.m.	Thorax opened	
12:32 p.m.	Blood from left ventricle just before it ceased beating	23

*Dog 11. 25 kilos. ♂*

12:00 m.	Ether anesthesia	
12:15 p.m.	Spinal fluid	56
12:25 p.m.	Blood from femoral artery	49
12:30 p.m.	Tracheotomy	
1:25 p.m.	Lymph from thoracic duct	47
1:25 p.m.	Blood from femoral artery	47

*Dog 12. 14 kilos. ♂*

11:00 a.m.	Chloroform anesthesia	
10:00 a.m.	Spinal fluid	52
10:07 a.m.	Blood from femoral vein	39
11:00 a.m.	Thoracic duct cannularized	
11:30 a.m.	Lymph taken	39
12:00 m.	Spinal fluid by occipito-atlantoid puncture	52
12:00 m.	Blood from femoral vein	38
2:30 p.m.	Lymph	32
2:35 p.m.	Spinal fluid	48
2:37 p.m.	Blood from femoral vein	33
4:00 p.m.	Spinal fluid	47
4:08 p.m.	Blood from femoral vein	29



*Dog 13. 30 kilos. ♂*

10:15 a.m.	Chloroform anesthesia	
10:20 a.m.	Tracheotomy	
10:25 a.m.	Left carotid connected to recorder	
10:30 a.m.	Spinal fluid.....	63
10:30 a.m.	Blood from femoral vein.....	39
10:33 a.m.	Started injection of 10 per cent ethyl alcohol in 0.9 per cent saline into left femoral. Chloroform dis- continued	
12:30 p.m.	Spinal fluid.....	53
12:30 p.m.	Blood from femoral vein.....	32
1:45 p.m.	Spinal fluid.....	48
1:45 p.m.	Blood from femoral vein, slight hemolysis.....	37
3:00 p.m.	Spinal fluid.....	46
3:00 p.m.	Blood from femoral vein.....	34

*Dog 14. 3 kilos. ♀*

10:00 a.m.	Ether anesthesia	
10:12 a.m.	Class demonstration (opened thorax)	
12:00 m.	Spinal fluid.....	42
12:00 m.	Blood from left ventricle.....	33
12:00 m.	Blood from right ventricle.....	32

*Dog 15. 12 kilos. ♂*

3:30 p.m.	Ether anesthesia	
3:40 p.m.	Spinal fluid.....	51
3:45 p.m.	Blood from femoral vein.....	45
3:50 p.m.	Intermittent faradization of sciatic	
5:15 p.m.	Blood from femoral vein.....	35
5:20 p.m.	Spinal fluid.....	44
6:20 p.m.	Injected 500 cc. of 5 per cent NaHCO <sub>3</sub> intravenous	
6:45 p.m.	Blood from femoral vein.....	115
6:50 p.m.	Spinal fluid.....	55
8:15 p.m.	Blood from femoral vein.....	104
8:20 p.m.	Spinal fluid (blood-stained).....	82
10:15 p.m.	Blood from femoral vein.....	101
10:20 p.m.	Spinal fluid (blood-stained).....	86
1:00 a.m.	Blood from the femoral vein.....	84
1:00 a.m.	Spinal fluid (blood-stained).....	77
1:10 a.m.	Died	

*Dog 16. 35 kilos. ♂*

12:30 p.m.	Morphine gr. 3 subcutaneous	
2:20 p.m.	Ether anesthesia	
2:25 p.m.	Spinal fluid.....	59
2:25 p.m.	Blood from femoral vein.....	51

2:40 to 3:20 p.m.	1500 cc. 5 per cent $\text{NaHCO}_3$ intravenous	
4:15 p.m.	Blood from femoral vein. ....	141
4:15 p.m.	Spinal fluid. ....	82
5:30 p.m.	Blood from femoral vein. ....	182
5:30 p.m.	Spinal fluid. ....	97
5:35 p.m.	Died	

*Dog 17. 15 kilos. ♀*

10:45 a.m.	Morphine gr. 2 subcutaneous	
11:15 a.m.	Ether anesthesia	
11:20 a.m.	Spinal fluid. ....	53
11:20 a.m.	Blood from femoral vein. ....	49
11:45 to 12:00 m.	500 cc. 5 per cent $\text{NaHCO}_3$ intravenous	
12:20 p.m.	Spinal fluid. ....	62
12:20 p.m.	Blood from femoral vein. ....	123
1:45 p.m.	Died	

*Dog 18. 14 kilos. ♂*

9:45 a.m.	Morphine gr. 2 subcutaneous	
12:00 m.	Ether anesthesia	
12:10 p.m.	Spinal fluid. ....	55
12:15 p.m.	Blood from femoral vein. ....	50
12:30 p.m.	Tracheotomy. Internal carotid connected to recorder	
1:00 p.m. to 1:15 p.m.	280 cc. of 5 per cent $\text{NaHCO}_3$ intravenous	
1:35 p.m.	Spinal fluid. ....	55
1:40 p.m.	Blood from femoral vein. ....	72
4:30 p.m.	Spinal fluid. ....	58
4:35 p.m.	Blood from femoral vein. ....	72
6:10 to 6:25 p.m.	500 cc. of 5 per cent $\text{NaHCO}_3$ intravenous	
7:00 p.m.	Spinal fluid. ....	66
7:00 p.m.	Blood from femoral vein. ....	115
10:00 p.m.	Spinal fluid. ....	74
10:00 p.m.	Blood from femoral vein. ....	92
1:10 a.m.	Spinal fluid. ....	80
1:10 a.m.	Blood from femoral vein. ....	102
1:25 a.m.	Died	

*Dog 19. 22 kilos. ♂*

10:30 a.m.	Ether anesthesia	
10:40 a.m.	Spinal fluid. ....	53
10:40 a.m.	Blood from femoral vein. ....	49
10:55 to 11:15 a.m.	500 cc. of 5 per cent $\text{NaHCO}_3$ intravenous	
11:40 a.m.	Blood from femoral vein. ....	104

*Dog 20. 12 kilos. ♂*

2:00 p.m.	Ether	
2:15 p.m.	Blood from femoral vein. ....	53
2:15 p.m.	Spinal fluid. ....	62

*Dog 21. 18 kilos. ♂*

11:00 a.m.	Morphine gr. 2	
11:30 a.m.	Etherized	
11:35 a.m.	Blood from femoral vein. ....	40
11:40 a.m.	Spinal fluid. ....	49

*Dog 22. ♀*

10:45 a.m.	Morphine gr. $\frac{1}{2}$	
11:00 a.m.	Etherized	
11:15 a.m.	Morphine gr. 2. Ether discontinued	
11:30 a.m.	Spinal fluid. ....	51
11:33 a.m.	Blood from femoral vein. ....	45

The CO<sub>2</sub> combining power of specimens of spinal fluid and blood plasma taken concurrently from dogs immediately after anesthesia had been accomplished, is shown in table 1. It will be noted that in all cases but one (dog 6) the CO<sub>2</sub> combining power of the venous plasma is less than that of the corresponding spinal fluid. The difference is quite marked, and varies from 3 to 27 volumes per cent. The CO<sub>2</sub> combining power of the arterial plasma only was determined in dogs 8 and 11. Assuming the CO<sub>2</sub> combining power of the venous plasma to be 4 volumes per cent higher than arterial plasma, there is an average difference of 7.5 volumes per cent in the CO<sub>2</sub> combining power of the venous plasma and the spinal fluid in the twenty-two experiments which have been carried out. The difference is greatest in those instances where chloroform was the anesthetic used, and least where morphine alone or morphine and ether were used.

As the CO<sub>2</sub> combining power of the venous plasma in all the human subjects examined was higher than that of spinal fluid, one hesitates to say that the CO<sub>2</sub> combining power of the spinal fluid of the normal dog is higher than that of the plasma. As Henderson (3) has pointed out, the decrease in the alkali reserve of the blood during anesthesia is primarily due to increased lung ventilation, and therefore to increased rate of CO<sub>2</sub> elimination. It is possible that the blood plasma in practically all our experiments had a lower CO<sub>2</sub> capacity due to increased lung ventilation prior to the time of sampling. There was as a rule more or less panting in dogs shortly after the injection of morphine but when the morphine injection preceded the etherization the latter was accomplished without any marked hyperpnoea being manifested. The alkali reserve of the spinal fluid of the normal animal therefore approximates that of the venous plasma but it is not necessarily identical with

it, while in the human subject the  $\text{CO}_2$  combining power of the spinal fluid is definitely lower than that of the plasma.

The differences which are noted in the alkali reserve of the spinal fluid and venous plasma of dogs anesthetized with chloroform compared with those anesthetized with ether are most striking. As there was even less struggle in the experiments where chloroform was used

TABLE 1  
*CO<sub>2</sub> combining power of plasma and spinal fluid. Concurrent specimens immediately after anesthesia*

DOG NUMBER	ANESTHETIC	SPINAL FLUID	VENOUS PLASMA	ARTERIAL PLASMA
1	Morphine only . . . . .	62	59	58
2	Morphine only . . . . .	61	52	
6	Morphine and ether . . . . .	60	62	
6	Morphine and ether 5 days later . . . . .	58	51	
6	Morphine and ether 2 days later . . . . .	52	44	
9	Morphine and ether . . . . .	66	61	
16	Morphine and ether . . . . .	59	51	
17	Morphine and ether . . . . .	53	49	
18	Morphine and ether . . . . .	55	50	
21	Morphine and ether . . . . .	49	40	
22	Morphine and ether . . . . .	51	45	58
3	Morphine and chloroform . . . . .	63	36	
7	Morphine, ether and chloroform (trace) . . . . .	66	61	
12	Chloroform . . . . .	52	39	
13	Chloroform . . . . .	63	39	
5	Ether . . . . .	54	49	49
8	Ether . . . . .	68		
10	Ether . . . . .	57	54	
11	Ether . . . . .	56		
15	Ether . . . . .	51	45	
19	Ether . . . . .	53	49	
20	Ether . . . . .	62	53	

than when ether was employed, and consequently less hyperpnoea, it would seem that the factor of increased  $\text{CO}_2$  loss emphasized by Henderson (3) is not the only one acting to cause a decrease in the alkali reserve of the blood. A possible cause of the greater drop in the  $\text{CO}_2$  combining power of the plasma under chloroform than under ether may be found in the difference in the degree of depression of the respiratory exchange in the tissue under these anesthetics. It would be theoretically possible, for example, to have a decrease in the alkali reserve as a

result of decreased  $\text{CO}_2$  production, and even decreased  $\text{CO}_2$  elimination, providing that the decrease in the former was greater than the decrease in the latter. Thus, as Benjamin Moore (4) has suggested, it may be possible for an actual decrease in the  $C_R$  to occur in shock, even though no increased lung ventilation be manifested, due as he expresses it to the respiratory pump working faster than the circulatory pump. If the rate of oxidation, and consequently the rate of  $\text{CO}_2$  production in the tissue be decreased, and the lung ventilation remains normal, then a decrease in the combining power of the plasma would occur. Another factor which may be very important is the appearance in the blood of unoxidized acid end products such as aceto-acetic acid and B-oxybutyric as a result of the disturbance of cell metabolism by the anesthetic.

TABLE 2

*$\text{CO}_2$  combining power of concurrent samples of spinal fluid, plasma and lymph*

DOG NUMBER	SPINAL FLUID	ARTERIAL PLASMA	VENOUS PLASMA	LYMPH
10			40	43
11		47		47
12	48		33	32

The  $\text{CO}_2$  combining power of spinal fluid, blood plasma and lymph taken concurrently, is shown in table 2. There is a very close agreement between the values obtained for plasma and lymph in all these experiments. The spinal fluid in dog 12 gave a much higher reading than either blood plasma or lymph. This is in accordance with other experiments of a similar nature. As the alkali reserve of the blood plasma had fallen considerably in dogs 10 and 12 before the samples were taken, it is to be concluded that the alkali reserve of blood plasma and thoracic duct lymph are in close accordance.

The rate of fall in the  $\text{CO}_2$  combining power of the blood plasma and spinal fluid with progressive shock due to prolonged anesthesia and trauma is recorded in table 3.

It will be noted in all the experiments quoted that alkali reserve of the spinal fluid never fell to a very low level even when the animal was at the point of death. The alkali reserve of both arterial and venous plasma however fell many points before death ensued. Henderson (5) found that 33 volumes per cent was the critical level for the  $\text{CO}_2$  combining power of the plasma. When (as a result of excess lung venti-

TABLE 3

*The rate of fall in the rate of CO<sub>2</sub> combining power of plasma and spinal fluid with progressive shock, as a result of prolonged anesthesia and trauma*

DOG NUMBER	TIME ELAPSED	SPINAL FLUID	VENOUS PLASMA	ARTERIAL PLASMA
	hours			
9	1½	66	61	46
	2½	58	50	
		49		
10	1½	57	54	
	2	53	40	
			23	
12	2	52	39	
	4½	52	38	
	6	48	33	
13	2	47	29	
	3½	63	39	
	4½	53	32	
15	2	48	37	
	3½	46	34	
	4½			
15	1½	51	45	
		44	35	

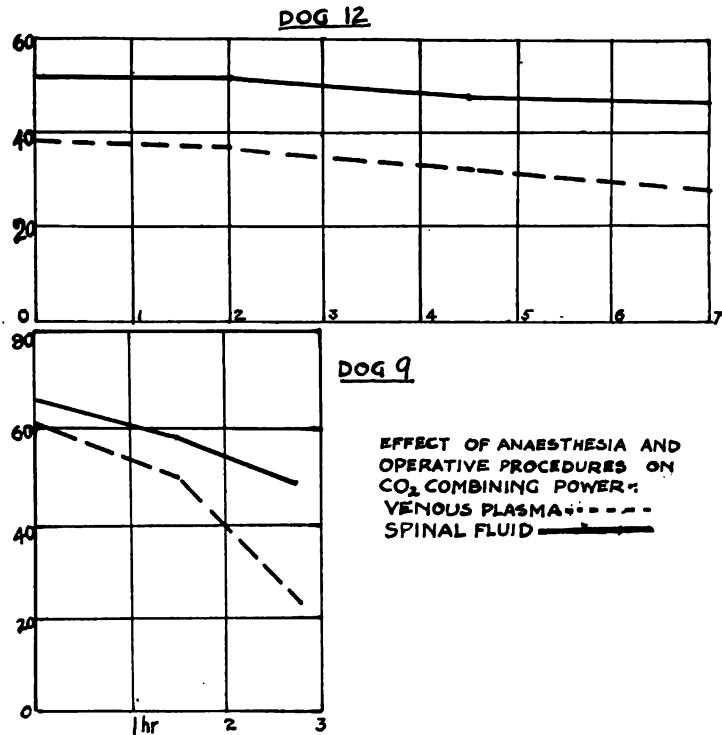


CHART 1

lation) the  $\text{CO}_2$  combining power of the blood fell below this level, animals did not recover, while a fall in the  $\text{CO}_2$  combining power to a level above 33 volumes per cent did not result in the death of the experimental animal. A steady and progressive fall in the alkali reserve of the plasma occurred in all cases, but in dog 13. (Chart 1, dogs 9 and 12.) In this latter case, the alkali reserve of the blood fell very sharply to 39 in the first few minutes as a result of chloroform

TABLE 4

*Rate of rise and fall in  $\text{CO}_2$  combining power of plasma and spinal fluid after  $\text{NaHCO}_3$  injection*

DOG NUMBER	TIME ELAPSED	SPINAL FLUID	VENOUS PLASMA
	<i>hours</i>		
15	{ 1/4 2 4 7	44	35
		55	115
		82	104
		86	101
		77	84
16	{ 1 2 1/4	59	51
		82	141
		97	132
17	{ 1/4	53	49
		62	123
18	{ 1/4 3 1/4 2nd injection 1/4 3 1/4 6 1/4	55	50
		55	72
		58	72
		66	115
		74	92
		80	102

anesthesia and trauma. At the end of two hours it had fallen to 32, while during the next hour and a quarter it rose to 37 and then fell to 34. The plasmas in this experiment showed a trace of hemolysis and this factor may account for the seemingly peculiar behavior of the alkali reserve of the blood in this case.

The fact that the alkali reserve of the spinal fluid remains at a comparatively high level even after shock has been manifested, and indeed even at the point of death, is most significant.

The rate of rise and fall in the  $\text{CO}_2$  combining power of the venous plasma and spinal fluid after intravenous injections of  $\text{NaHCO}_3$  is shown in table 4. The maximum  $\text{CO}_2$  combining power of the plasma would of course occur immediately after the bicarbonate injection. In order to give time for equilibrium to be established between the plasma and spinal fluid concurrent samples of these latter were not taken until twenty to sixty minutes had elapsed after the injection was completed.

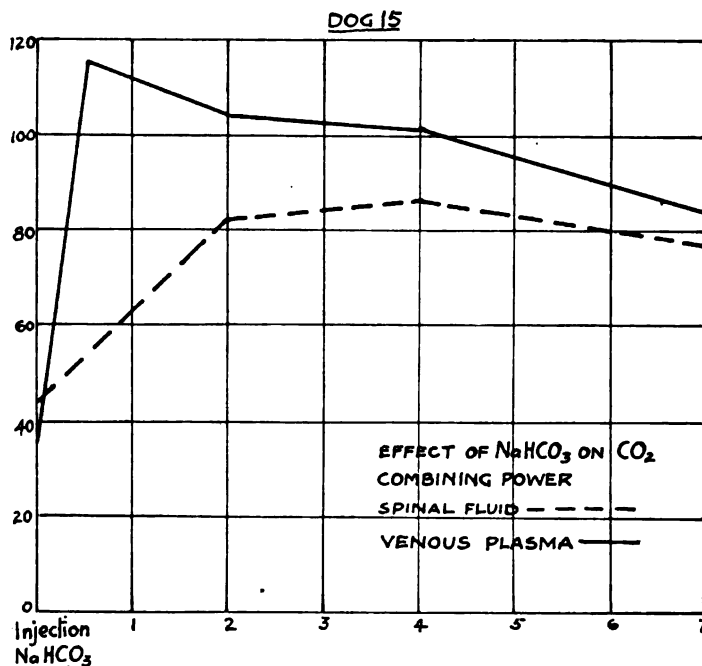
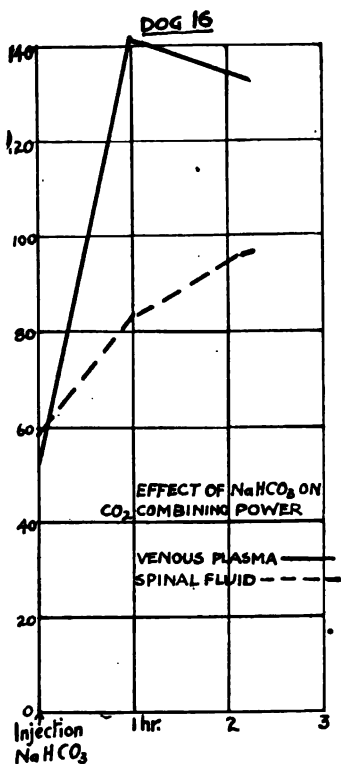
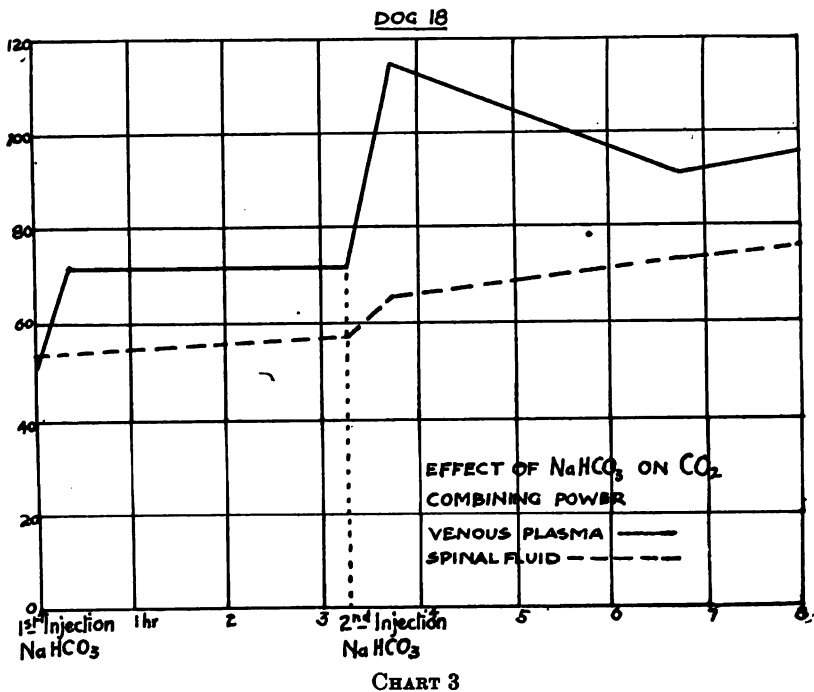


CHART 2

The alkali reserve of the plasma of dog 15 (chart 2) was brought to a low level before the bicarbonate solution was injected. One-half hour after the injection of 500 cc. of 5 per cent  $\text{NaHCO}_3$  was completed, the  $\text{CO}_2$  combining power of the plasma was more than tripled while that of the spinal fluid had risen only 25 per cent or from 44 to 55 as against a rise in the plasma of from 35 to 115. After two hours the plasma bicarbonate had fallen to 104 while that of the spinal fluid was 82. Four hours after the injection the plasma value was 101 while the spinal fluid  $\text{CO}_2$  combining power was at its maximum 86 for this experiment.





The spinal fluid  $\text{CO}_2$  combining power then fell slowly to 77 and that of the blood plasma to 84. The blood pressure was maintained for some hours at a high level. During the last half-hour however it fell rather abruptly and respiratory failure followed soon after. Experiments 16, 17, 18 and 19 (chart 3) show somewhat similar results. The spinal fluid bicarbonate rises very slowly, a great gap between it and that of the plasma being maintained. In dog 18 (chart 4) the alkali reserve of the spinal fluid rose in three and one-fourth hours after the first injection of  $\text{NaHCO}_3$  from 55 to 58 while the blood plasma reserve was 72 twenty minutes after the injection and this level was maintained for the three hours following. It would appear in this instance that equilibrium had been established between the plasma and the spinal fluid and that an actual difference of fourteen points was being maintained. After the plasma bicarbonate had been raised to a much higher

TABLE 5  
*CO<sub>2</sub> combining power of venous plasma and spinal fluid in man*

SUBJECT	CONDITION	SPINAL FLUID	ARTIC FLUID	VENOUS PLASMA
1 T. B. T.	Hysteria	48	56	55
2 T. C.	Hepatic cirrhosis	46		58
3 N. H. P.	Normal	60		69
4 R. B. R.	Normal	56		63
5 J. B. A.	Under morphine $\frac{1}{4}$ gr., ether, atropine $\frac{1}{16}$ gr. Osteo- myelitis of femur	49		58

level, the spinal fluid bicarbonate value rose slowly reaching a maximum of 80 just before death ensued after six and three-fourths hours.

The respirations were much shallower after the sodium bicarbonate had been injected in those instances where morphine was given subcutaneously prior to anesthetization.

The blood pressure rose slightly after the bicarbonate injection due to the osmotic effect and it was maintained at a high level for very long periods. Death occurred suddenly in all instances, the blood pressure failing first and respiration shortly thereafter (chart 5). Artificial respiration, heart massage and intravenous injection of acid phosphate were without effect. Definite stimulation of respiration both as regards rate and amplitude was obtained in dog 19 during and after the injection of  $\text{NaHCO}_3$ . This animal had received ether only. This would be

in accordance with the observations of Hooker, Wilson, and Connet (6) and Scott (7) that the respiratory center may be stimulated by increased  $\text{HCO}_3$  ion concentration more or less independent of the H-ion concentration of the blood.



CHART 5

#### DISCUSSION

The  $\text{CO}_2$  combining power of the plasma is not an absolute index of the alkali reserve of all body fluids. The experiments herein reported indicate that there is a very close agreement between the alkali reserve

of blood plasma and lymph. There may also be a very close agreement in the alkali reserve of blood plasma and spinal fluid. The fact, however, that the  $\text{CO}_2$  combining power of the spinal fluid does not fall parallel with that of the blood plasma during shock but tends always to maintain a relatively high level, taken in conjunction with the observation that a high level of sodium bicarbonate in the blood does not produce an equally high level for the latter in the spinal fluid, would lend a measure of support to the view of Halliburton (8) that the spinal fluid is the lymph of the brain. It would appear that the tissues of the brain and spinal cord are afforded an extra measure of protection against either an acidosis or an alkalosis of the blood. The  $\text{C}_\Sigma$  of the spinal fluid probably tends to remain constant longer even than the blood during a condition of developing acidosis or alkalosis. Our results may however be interpreted in the light of the theory that the spinal fluid is more or less inert. Sudden changes in the concentration of a blood constituent may not be effective in producing like changes in the concentration of the same constituent in the spinal fluid due to slow or difficult diffusion between the membranes separating the fluid from the plasma. The authors favor the former interpretation of the results of the experiments quoted.

#### SUMMARY

1. The alkali reserve of concurrent specimens of blood plasma, lymph and spinal fluid has been determined.
2. The  $\text{CO}_2$  combining power of the plasma is an approximate index of the "alkali reserve" of the body fluids of the normal animal, as has been claimed by Van Slyke.
3. The  $\text{CO}_2$  combining power of the spinal fluid as compared with that of the plasma is maintained at a relatively high level in shock.
4. The  $\text{CO}_2$  combining power of the spinal fluid tends to remain at a comparatively low level when the  $\text{NaHCO}_3$  content of the blood is increased experimentally.
5. It would appear that the  $\text{C}_\Sigma$  of the spinal fluid is protected to a greater degree than that of the blood.

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THE EFFECT OF PROLONGED HYPERPNOEA ON THE CARBON DIOXIDE COMBINING POWER OF THE PLASMA, THE CARBON DIOXIDE TENSION OF ALVEOLAR AIR AND THE EXCRETION OF ACID AND BASIC PHOSPHATE AND AMMONIA BY THE KIDNEY

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INTRODUCTION

It has been shown by Leathes (1) that the amount of acid as compared with basic phosphate in the urine can be materially altered by prolonged hyperpnoea. The method of double titration using two indicators, phenolphthalein and methyl-orange, described by him, furnishes a very simple, yet approximately accurate method whereby one can follow sudden changes in the rate of excretion of total phosphate, and also the relative amounts of the acid and the basic types.

Henderson and Haggard (2) have shown that by increasing or decreasing the rate of carbon dioxide elimination at the lung surface in dogs, the carbon dioxide content as well as capacity of the blood is decreased or increased respectively, the ratio  $\frac{\text{CO}_2 \text{ dissolved}}{\text{CO}_2 \text{ combined}}$  tending to remain a constant. This change in the carbon dioxide combining power of the blood, as Henderson and Haggard (2) have pointed out, indicates that the tissues can either take up alkali from the blood or give up alkali to it, as occasion demands. Henderson, Prince and Haggard (3) found that voluntary hyperpnoea of comparatively short intervals caused a decrease in the venous return to the heart, while systolic, diastolic and pulse pressures were not greatly affected. Henderson (4), Haldane and Poulton (5) and many others have observed marked disturbances of varied character as a result of forced breathing.

The results of a series of experiments carried out upon several members of the medical student body in the University of Alberta, who

kindly offered their services, are reported in this paper. These experiments were designed to follow the changes produced in the carbon dioxide tension of the alveolar air, the carbon dioxide combining power of the venous plasma, the acidity of the urine, and in the rate of excretion of phosphate, ammonia and water by the kidneys, by prolonged voluntary hyperpnoea. A number of observations were also made on blood pressure, pulse rate, hemoglobin and white blood cell content of the blood of these subjects before, during, and after periods of hyperpnoea, while in one instance the percentage of blood sugar was determined before and after a period of forced breathing.

The production of an alkaline urine by a normal individual with a low carbon dioxide combining power of the plasma is of interest. Palmer and Van Slyke (6) have found that in normal men the urine becomes more alkaline than the blood when the  $\text{CO}_2$  held as plasma bicarbonate exceeds  $71 \pm 5$  volumes per cent. In prolonged hyperpnoea one finds that the kidney responds in much the same manner as it would if the subject had received a large dose of sodium bicarbonate by mouth or by intravenous injection. The  $\text{C}_2$  of the blood is the essential factor in the regulation of this phase of renal activity irrespective of what the bicarbonate level may be.

#### METHODS

Immediately before the initiation of the period of hyperpnoea the subject emptied his bladder, provided a sample of venous blood and a sample of alveolar air. When the period was about to be brought to a close a sample of venous blood and of alveolar air was again taken, the subject emptying the bladder within a few minutes. Further samples of venous blood and alveolar air were taken concurrently fifteen to twenty minutes after, and forty to one hundred and eighty minutes after the cessation of the period of hyperpnoea. The urine in certain cases was taken again as soon as a sufficient quantity had been secreted, usually within an hour. It was also taken in a few cases at intervals of about two hours for a subsequent period.

Blood pressure was taken by the auscultatory method, using either the aneroid type of recorder or the mercury column. Hemoglobin was determined colorimetrically by the acid hematin method, using the Duboscq colorimeter (7). White blood cell counts were made in the usual manner.

The carbon dioxide content of the alveolar air was determined by the method of Fridericia (8) while the  $\text{CO}_2$  combining power of the venous plasma was determined by the method of Van Slyke and Cullen (9). The blood was aspirated from an arm vein into a glass syringe containing a trace of powdered potassium oxalate. It was immediately centrifuged and the plasma saturated with carbon dioxide at the alveolar tension of the normal subject. Duplicate analyses were made in practically all instances. The readings were reduced to cubic centimeters of carbon dioxide at  $0^\circ\text{C}$ . and 760 mm. bound by 100 cc. of plasma at the respective temperatures.

The ammonia in the urine was determined by the formalin method of Malfatti (10), and calculated in terms of milligrams per 100 cc. of urine and milligrams secreted per hour.

The acidity of the urine was determined according to the titration method of Leathes (1) and represents simply the ratio of acid to basic phosphate.

The amount of N/10 acid, multiplied by 10, required to carry 10 cc. of urine previously made neutral to phenolphthalein to the methyl-orange point, has been taken as an approximate index of the total phosphate, for in such a titration all of the phosphate is first made basic in type and then converted to the acid form. This value is exaggerated slightly in the case of the urine secreted during the stage of hyperpnoea, due to the fact that an appreciable amount of carbonate is present in such a sample. The rate of phosphate elimination per hour was calculated from the data so obtained.

The rate of the secretion of urine was calculated on the basis of cubic centimeters per hour for periods before, during and after hyperpnoea.

Blood sugar was determined by the Lewis Benedict method (11).

The experiments were carried out at different times of the day, the duration of the period of forced breathing varying from fifteen to forty minutes. The results obtained show practically the same types of variations, irrespective of the time the experiments were carried out.

#### RESULTS

The results of several experiments on various subjects are shown in tabular form in the accompanying tables. Two protocols which are more or less typical of all the others are also shown. A marked diuresis occurred during the period of the experiment in nearly every instance. In the case of J. A. P. where it seemingly did not appear, the sample



voided immediately before the experiment was a four-hour specimen (8:00 a.m. to 12:00 noon), and doubtless secreted in a period during which the morning diuresis would be manifested. The rate of secretion per hour recorded for the period previous to the experiment when such calculation is based on the volume of a sample secreted over a period of some hours is not necessarily therefore a true index of the rate of secretion for the hour immediately preceding the hyperpnoea. The diuresis continued for a few minutes following the cessation of the forced breathing, then the rate of the secretion fell off sharply.

The tension of carbon dioxide in the alveolar air varied from 39.5 mm. in W. A. R. to 50.9 mm. in W. N. S., which represents approximately the range of variation within the normal. After a period of twenty to forty minutes forced breathing the tension of  $\text{CO}_2$  in the alveolar air fell to 16 mm. in case of P. E. L. and 28.8 mm. in subject E. S. S. The average decrease in the carbon dioxide tension of the alveolar air in fifteen experiments was 44 per cent.

It was found in those cases in which a determination of the alveolar carbon dioxide tension was made at intervals after the cessation of hyperpnoea, that there was a return to within 4 to 6 mm. of the original tension in from fifteen to twenty minutes, while after longer intervals the alveolar carbon dioxide tension was usually equal to or greater than that existent prior to the experiment. We do not wish to draw any inferences, however, when only small differences in tension are recorded because of the fact that the Fridericia apparatus can only be relied upon to give approximate results.

The carbon dioxide combining power of the venous plasma, taken prior to the period of forced breathing showed about the normal range of variation the average in the fifteen experiments being represented by the factor 66.5. The ratio  $\frac{\text{CO}_2 \text{ dissolved}}{\text{CO}_2 \text{ combined}}$  calculated on the basis of the solubility coefficient of carbon dioxide being 0.52 shows but little deviation from the mean of 0.0470.

The carbon dioxide combining power of the venous plasma immediately after, or during the last few moments of the period of increased lung ventilation, showed in every instance a marked decrease, the average fall being 14.3 per cent. The sudden return of this factor to the normal value was most striking. This was practically complete in from one to one and a half hours after the hyperpnoea had ceased.

The titratable acidity of the urine fell very abruptly with the onset of hyperpnoea. This was due to the increase in the rate of secretion of

basic, as opposed to acid phosphates, as has been shown by Leathes (1). There was not only a relative increase in the amount of basic phosphates, but there was also an actual increase in the rate of excretion of total phosphates, as well as an actual increase in concentration of the phosphates in the urine. In one instance (E. D. E.) the urine voided immediately after the cessation of hyperpnoea was alkaline to phenolphthalein, 10 cc. requiring 0.05 cc. of  $\frac{N}{10}$  acid to make it neutral to this indicator.

The titratable acidity of the urine was low for one to two hours after the experiment, then it rose rather sharply. The total phosphate excretion was lower in the period immediately succeeding the experiment, and the degree of concentration of the same was also less.

Throughout the period of forced breathing the excretion of carbonate was marked but the actual amount secreted was not large.

The concentration of ammonia in the urine fell during the experimental period in all instances. The fall was very marked in some, but less marked in others.

The actual excretion rate of ammonia was always less during hyperpnoea.

The hemoglobin determination, when made, indicated little or no fluctuation in blood volume as a result of the experiment.

The white blood cell counts point to a mild leucocytosis as a result of the forced breathing.

The pulse pressure was decreased as a result of hyperpnoea. This was due to a decreased systolic and an increased diastolic pressure. The change in the character of the pulse, as determined by the palpatory method, was most marked. The actual pulse rate was decreased in some subjects and increased in others.

The blood sugar was found to be increased in one instance where this factor was determined.

Chart 1 illustrates the changes noted in subject E. C. M. as a result of thirty-four minutes hyperpnoea.

The symptoms manifested by the different subjects were for the most part more or less similar, differing only in degree. Objectively the face was flushed in some subjects, it showed little change in others, while in still others both the face and hands became pallid. Muscle tonus was increased in all instances and in a few subjects definite tetany was observed. The muscles affected in these latter instances were those of the fore-arm and hand and to a less extent the muscles of the face and leg. Profuse sweating was noted in a small group of subjects.

Subjectively the individual experienced a tingling sensation very soon after hyperpnoea was commenced. This was localized in the fingers, toes and teeth, and was followed soon after by numbness in the extremities which in a few instances amounted to almost complete cutaneous anesthesia. Most subjects showed a tendency to become drowsy and all were more or less dizzy especially if they attempted to walk. Frontal headache developed in a few subjects. None of our subjects complained of nausea but two remarked that they were unusually hungry and thirsty after their experiment.

## PROTOCOLS

*J. A. P.*

- 12:00 m. Urine 225 cc. 4-hour sample, 102 mgm.  $\text{NH}_3$  per 100 cc. 62 per cent acid.  
12:05 p.m. Systolic pressure 112 mm., diastolic 64, pulse 64.  
12:15 p.m. Alveolar air,  $\text{CO}_2$  = 5.7 per cent  
12:17 p.m. Blood from arm vein  $\text{CO}_2$  combining power of plasma by Van Slyke method 65  
12:17 p.m. to 12:50 p.m. Hyperpnoea  
12:50 p.m. Alveolar air  $\text{CO}_2$  = 2.8 per cent  
12:54 p.m. Blood from arm vein  $\text{CO}_2$  of plasma 54  
1:00 p.m. Urine 52 cc. 15 mgm.  $\text{NH}_3$  per 100 cc. 5 per cent acid  
1:05 p.m. Systolic pressure 108, diastolic 80, pulse 70  
1:14 p.m. Blood from arm vein,  $\text{CO}_2$  of plasma 61  
1:15 p.m. Alveolar air,  $\text{CO}_2$  = 5.8 per cent  
3:15 p.m. Blood from arm vein  $\text{CO}_2$  of plasma 65  
3:20 p.m. Urine 55 cc., 76 mgm.  $\text{NH}_3$  per 100 48 per cent acid

*E. C. M.*

- 3:30 p.m. Urine 100 cc. 1½-hour sample, 75 mgm.  $\text{NH}_3$  per 100 59 per cent acid  
3:35 p.m. Alveolar air,  $\text{CO}_2$  = 6.1 per cent  
3:40 p.m. Blood from arm vein,  $\text{CO}_2$  of plasma = 64  
3:10 p.m. to 4:14 p.m. Hyperpnoea  
4:10 p.m. Alveolar air,  $\text{CO}_2$  = 2.3 per cent  
4:13 p.m. Blood from arm vein,  $\text{CO}_2$  of plasma = 56  
4:15 p.m. Urine 100 cc. 4 mgm.  $\text{NH}_3$  per 100 cc. 2.3 per cent acid  
4:27 p.m. Blood from arm vein,  $\text{CO}_2$  of plasma = 61  
5:15 p.m. Urine 50 cc., 29 mgm.  $\text{NH}_3$  per 100 6.7 per cent acid  
5:25 p.m. Blood from arm vein,  $\text{CO}_2$  of plasma = 63  
5:27 p.m. Alveolar air,  $\text{CO}_2$  = 6.4 per cent  
7:05 p.m. Urine 110 cc. 65 mgm.  $\text{NH}_3$  per 100 cc. 44 per cent acid  
9:00 p.m. Urine 220 cc. 34 mgm.  $\text{NH}_3$  per 100 cc. 52 per cent acid  
11:35 p.m. Urine 250 cc. 29 mgm.  $\text{NH}_3$  per 100 cc. 22 per cent acid

TABLE 1

	J.A.P. 12:00 NOON	E.N.S. 3:30 P.M.	B.S.B. 3:00 P.M.	W.C.C. 4:30 P.M.	E.C.M. 3:30 P.M.	W.A.R. 10:40 A.M.	P.A.L. 4:00 P.M.	G.T.Y. 4:00 P.M.	F.E.L. 3:30 P.M.	F.L.B. 12:45 P.M.	E.D.E. 11:50 A.M.	J.L.J. 4:00 P.M.	C.N.G. 3:10 P.M.	J.G.W. 3:20 P.M.	C.G.W. 11:25 A.M.	F.E.L. 4:30 P.M.	P.E.L. 5:00 P.M.
Duration of hyperpnoea (minutes).....	37	38	35	33	34	32	38	22	32	20	40	33	25	20	23	15	15
Secretion urine, cc. per hour, 1 to 4 hours before.	56	50	50	40	66	46	47	144	28	56	35	100	150	90	56		
During hyperpnoea.....	52	113	84	88	133	220	107	147	100	100	77	172	223	90	90		
After 1 to 2½ hours.....	24	46	84		50								137				
After 2½ to 6 hours.....			34	58	83								35				
Per cent of CO <sub>2</sub> in alveolar air 0° and 760 mm. before.	5.7	6.7	6.4	6.7	6.1	5.2	6.6	6.4	5.9	5.8	5.7	5.6	5.6	5.9	6.1		
After hyperpnoea.....	2.8	3.3	3.8	3.2	2.3	3.1	3.1	2.7	2.1	2.7	2.6	2.7	3.0	3.7	2.8		
15 minutes to 20 minutes after.....	5.8	6.2	5.7	5.9	5.8								5.6				
40 minutes to 80 minutes after.....			5.9		6.4												
Alveolar CO <sub>2</sub> tension before.....	43.3	50.9	48.6	50.9	46.3	39.5	50.1	48.6	44.8	44	43.3	42.5	44.8	42.5	46.3		
After.....	21.3	25	28.8	24.3	17.5	23.6	23.6	20.5	16.0	26.5	20.0	20.5	22.8	28.1	19.3		
CO <sub>2</sub> combining power with plasma before hyperpnoea	65	70	73	69	64	65	65	67	65	65	65	65	65	68	69		
Immediately after.....	54	62	60	60	56	57	56	51	57	55	59	57	56	56	56		
15 minutes to 20 minutes after.....	61	67	69	62	61		63										
40 minutes to 180 minutes after.....	65				63				67						67		
Acidity of urine before hyperpnoea.....	62	67	32	25	59	23	58	26	65	48	43	32	63	11	44		

Immediately after .....	5	1.6	3	2.5	2.3	5.5	15	1	3	12	0	3	6	10	
After 1 to 2 hours.....	48	22	8		8								8		
After 2 to 6 hours.....		76	40	50	44								56		
Ammonia excretion mgn.															
per hr. before .....	56	50	22	20	49	15	39	49	40	29	22	52	26	19	
During .....	8	15	10	8	5	13	20	7	25	9	6	24	22	13	
After .....	18	23	17	35	26								31		
Ammonia excretion mgn.															
per 100 cc. before .....	102	99	44	51	75	32	82	34	143	52	62	52	17	34	
During .....	15	17	12	9	4	6	22	5	25	9	8	14	10	22	
After .....	76	45	19	60	47								33	14	
Phosphate excretion per hour															
before, phenolphthalein															
to methyl-orange .....	45	33	20	33	46	21	22	63	21	31	26	50	13.5	47	
During .....	48	141	97	141	173	130	61	150	124	73	127	189	75	133	
After .....	21	30	54	35	37								55		
CO <sub>2</sub> dissolved before	0.0456	0.0497	0.0456	0.0504	0.0495	0.0415	0.0527	0.0496	0.0472	0.0465	0.0456	0.0448	0.0472	0.0459	
CO <sub>2</sub> as bicarbonate															
After .....	0.0288	0.0267	0.0328	0.0277	0.0206	0.0283	0.0288	0.0275	0.0191	0.0255	0.0229	0.0246	0.0279	0.0260	
Blood pressure before {S.	112												140	134	
hyperpnoea ..... {D.	68												64	80	
After ..... {S.	108												125	130	
After ..... {D.	80												82	86	
Pulse before .....	64												98	82	
Pulse after .....	70												82	92	
White blood cells before .....															
White blood cells after .....															
Blood sugar before .....															
Blood sugar after .....															

8,000 8,500  
7,600 11,200  
10,300 11,500  
0.13  
0.20

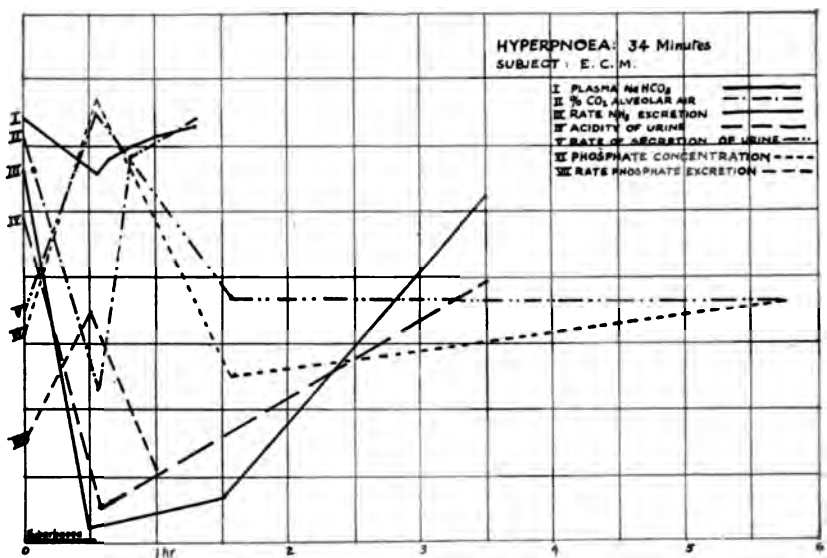


CHART 1

## DISCUSSION

The changes produced in the carbon dioxide combining power of the blood and in the rate of excretion of phosphate, ammonia and acid by the kidney, by forced breathing, and the various symptoms noted during hyperpnoea, are interpreted by us as due to the production of a mild degree of alkalosis.

The "washing out of carbon dioxide" from the blood tends to decrease the normal ratio  $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$  (12), by decreasing the numerator.

This would indicate a decrease in the H-ion concentration of the blood. The kidney responds at once by excreting a less acid, or even an alkaline urine (E. D. E.), at the same time the rate of secretion is increased, and a small quantity of sodium bicarbonate is excreted. These facts would favor a mechanical interpretation of kidney function.

Van Slyke (9) has suggested that the alkali reserve of the blood plasma may be taken as an index of the alkali reserve of the whole body. If this were always so it would appear in the experiments reported in this paper that a great decrease in the alkali reserve of the body had occurred. This however is obviously not the case, or the prompt re-

turn of the blood bicarbonate to its normal level within a few minutes after the cessation of hyperpnoea would not be possible.

Again the kidney, while excreting larger quantities of alkali during the experimental periods, does not eliminate sufficient to account for the fall in blood bicarbonate observed. The reaction of the blood is protected to a certain degree during the stage of "washing out of carbon dioxide" by increased excretion of alkali by the kidney. What is most important, however, is the buffer action of the tissue. The tissue soaks up alkali, as Henderson (2) has shown, and in so doing must change its own reaction to an appreciable extent. The alkali which is taken up by the tissue during the stage of low carbon dioxide tension in the blood, is apparently returned to the blood as the carbon dioxide tension of the latter rises following the cessation of hyperpnoea.

Tileston (13) has reported severe tetany in a case of Weil's disease following intravenous injection of sodium bicarbonate solution producing a plasma carbon dioxide combining power of 80 volumes per cent.

We have injected large doses of 5 per cent sodium bicarbonate in distilled water intravenously into dogs under local as well as general anesthesia, but in two cases only has the least sign of tetany been manifested. The dog may however have a greater tolerance for bicarbonate than the human subject.

The ratio  $\frac{\text{CO}_2 \text{ dissolved}}{\text{CO}_2 \text{ combined}}$  which has been determined for all subjects prior to and after the experiment from the data furnished is not in the latter instance a reliable index, as the carbon dioxide actually held in combination as bicarbonate in the venous blood would be several points lower than shown in the table, the plasma in all instances having been saturated with alveolar air from the normal subject. When the true value for  $\text{CO}_2$  combined in the venous plasma of the subject at the end of an experiment was determined, it was found that the ratio  $\frac{\text{CO}_2 \text{ dissolved}}{\text{CO}_2 \text{ combined}}$  was still much below normal, thus indicating that an actual decrease in H-ion concentration of the blood had occurred.

Unless there are acids other than carbonic which appear in the blood concomitant with the "washing out of carbon dioxide," the hydrogen ion concentration must be decreased by hyperpnoea. The urine was examined in several instances for aceto-acetic acid but none was found. No examination was made for lactic acid in blood or urine. This latter substance has been shown by Macleod (14) to appear in the urine in increased amount after alkali administration. It is possible that it

might be a compensating factor in these experiments. The decrease in the rate of ammonia elimination observed is in keeping with previous work on the significance of the ammonia factor. We have found that the decrease in the rate of secretion of ammonia is not at all uniform. The excretion is markedly suppressed in some cases, while in others the rate of elimination is only slightly depressed.

The exact cause of the hyperglycemia noted in P. E. L. was not determined.

The leucocytosis noted in some subjects as a result of hyperpnoea is due probably to the mechanical factor involved. The lymph nodes in the thorax are directly stimulated by the excessive respiratory movements and as a result give up white cells. It was found in a control experiment on a dog that no increase in the white cell count resulted after the intravenous injection of  $1\frac{1}{2}$  g. of sodium bicarbonate per kilo.

The development of definite tetany and "muscle cramps" in many subjects during hyperpnoea appears to us of great significance. These manifestations are due, it would appear, to an alkalosis of the tissue.

These observations would lend support to the view of Wilson (15) and others that parathyroid tetany is a form of alkalosis.

It is possible that the development of "cramp" in swimmers and also in runners may be due to a tissue alkalosis. The increased lung ventilation, in both swimmers and runners, may result at times in a proportionally greater carbon dioxide loss than production and if such a condition did occur then a tissue alkalosis might result. Ether spasm may also be due to a temporary alkalosis resulting from hyperpnoea.

#### SUMMARY

1. The effect of prolonged voluntary hyperpnoea in normal adults on the carbon dioxide tension of the alveolar air, the carbon dioxide combining power of the venous plasma, the rate of excretion of water, ammonia, basic and acid phosphates by the kidney has been determined.

2. The average fall in the  $\text{CO}_2$  tension of the alveolar air was 44 per cent.

3. The average fall in the  $\text{CO}_2$  combining power of the venous plasma was 14.3 per cent.

4. The acidity of the urine was markedly decreased.

5. A diuresis was noticed.

6. The rate of the elimination of phosphates was increased.

7. The rate of ammonia excretion was suppressed.



8. A leucocytosis was observed.
9. Hyperglycemia was produced.
10. Typical symptoms were manifested.
11. It is suggested that muscle "cramp" and ether spasm may be due to a temporary alkalosis.

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## IS THE LUMINESCENCE OF CYPRIDINA AN OXIDATION?

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In two papers, published in the January number of this Journal, Kanda (1) has criticised my work (2) on *Cypridina hilgendorfi* and has come to the conclusion that the luminescence of this animal is not an oxidation. Before showing that this is incorrect I wish to discuss several points which Kanda has brought up. While I think it is always unfortunate to engage in controversial communications, the relation of oxygen to luminescence is so fundamental that I feel, in the interest of truth, it is necessary to answer his criticism. I trust that the methods of removing oxygen herein described may be of value to physiologists in general.

First I call attention to the fact that I have dropped the words photogenin and photophelein as used in the paper which Kanda criticises and have adopted Dubois' terminology, luciferase and luciferin, and believe Dubois' interpretation is correct. My reasons for this are given in a paper (3) which clears up many of Kanda's criticisms, published in November, 1918, fourteen months before Kanda's paper appeared and twelve months before Kanda's paper was accepted for publication.

Second, Kanda remarks that I am "misled" in the use of the terms "luminous organ," "luminous gland," etc., and did not realize that the luminous material must be extruded from the gland cell, before the luminescence appears. I have always realized this and on page 320 (2) state that "these" (granules in the gland cells) "are extruded and dissolve to a colorless solution, absolutely free of visible granules, which gives the light." Students of animal light use the word luminous gland whether the luminescence is intracellular or extracellular. Although I am credited with giving a "full account of the maxillary gland" by Kanda I never intended to do this but referred for structural peculiarities to Professor Yatsu's paper (4), which appeared later in the *Journal of Morphology* and the proof of which I corrected. I am

quite aware that two different secretions are formed in the luminous gland. In fact three different types of granules can be observed in the gland of the living animal and Dahlgren (5) has described, from stained sections, four different secretions.

Third, on page 557 (1), referring to protein tests on the luminous material, there is evidently some bad proof reading, but in addition, by not quoting me fully, Kanda gives the impression that since I obtained negative results with protein tests I believe the luminous materials of *Cypridina* are not of protein nature. What I said, that Kanda omits, is, "I do not mean to infer from this" (negative biochemical tests) "that the luminous substance is neither protein, fat, nor carbohydrate but merely that the concentration giving a bright light is too small to respond to chemical tests" (2, p. 322). If Kanda will examine my paper (6) on "The chemical nature of *Cypridina* luciferin and *Cypridina* luciferase," which appeared twelve months before his paper, he will find the chemical nature of *Cypridina* luciferin and luciferase fully discussed.

Kanda evidently regards his discovery that the production of light by *Cypridina hilgendorfi* is not an oxidation a most important one, as he devotes a separate paper to this subject (1, p. 561). It would be important if it were true, but it is not true. A very low pressure of oxygen is sufficient to cause luminescence and these traces of oxygen must have been present in Kanda's experiments. Briefly, Kanda's experiment is as follows: He placed dried *Cypridina* powder in a bottle connected with a vacuum pump, a gas generator and a flask of oxygen-free water in such a way that the air in the bottle could be evacuated and then replaced by water saturated with  $H_2$ ,  $N_2$  or any other gas to be investigated. Rubber stoppers and rubber-glass joints were used. He obtained luminescence when the oxygen-free (supposedly) water is allowed to come in contact with dry *Cypridina* powder in the oxygen-free (supposedly) bottle, and he consequently concluded that no oxygen is necessary for luminescence. This method is faulty in two respects.

First I must point out that it is an exceedingly difficult matter to remove the last traces of oxygen from a gas or from water and to keep the oxygen away, and that it is practically impossible to do this with the cumbersome and complicated apparatus which Kanda uses (1, p. 562). Air will leak in around rubber-glass joints especially when subjected to a vacuum, no matter how tight they may appear. I long ago discarded rubber-glass connections where apparatus must be evacuated and then filled with pure gas. The only way to keep air

from leaking into an apparatus is to have all glass connections or make joints of lead tubing and deKotinsky cement. A small amount of oxygen is present in Kanda's apparatus and this is sufficient to give the luminescence he has observed. I would advise Kanda to test his apparatus by placing a small piece of phosphorus in bottle E and determining if its luminescence ceases under the same conditions in which the luminescence of *Cypridina* continues. The phosphorus must not, of course, be entirely covered with water. The luminescence of phosphorus requires only a very low oxygen pressure and for some time it was thought not to be an oxidation but the earlier investigators did not realize how low is the oxygen pressure necessary for luminescence. It should also be remembered that at ordinary temperatures phosphorus does not luminesce in pure oxygen. Nevertheless the luminescence of phosphorus is the result of an oxidation.

The second source of error is due to the fact that one can never remove all the oxygen from dried *Cypridina* with an air pump. There is some gas present in the dried tissue and if this tissue is moistened with absolutely oxygen-free water, light will result. I cannot attempt to explain how this gas is held in the dried tissue, whether absorbed, chemically bound or mechanically entangled, but it is there. The experiment which has led me to the above conclusion will be found at the end of this paper, after experiments which show the dependence of *Cypridina* luminescence on oxygen have been described.

The following experiment shows conclusively that the luminescence of *Cypridina* is an oxidation and requires the presence of free oxygen. The vessel, A (fig. 1), is partly filled through stopcock *a* with a filtered luminescent aqueous solution of luciferin and luciferase. If the luciferin is concentrated and the luciferase dilute, the luminescence will be bright and last with very gradually fading intensity for 20 or more minutes. Tube B, which is sealed to A with all glass connections through stopcock *b*, contains a sealed-in platinum spiral which can be made to glow by passing an electric current through it. Hydrogen gas from a cylinder is passed over the glowing spiral and through the luminescent solution. Any oxygen which the hydrogen contains is burned to water. This is a much simpler and more efficient way of removing the oxygen than absorption in alkaline pyrogallol. By connecting *a* to an air pump and properly turning the stopcocks *a* and *b*, vessel A can be alternately evacuated and filled with hydrogen. Three or four repetitions of this procedure are sufficient to render the luminescent solution in vessel A free of oxygen and the light disappears.

By turning off the platinum glower, a small amount of oxygen can now be admitted along with the hydrogen, and, allowing this hydrogen, which may contain less than  $\frac{1}{2}$  of 1 per cent of oxygen, to bubble

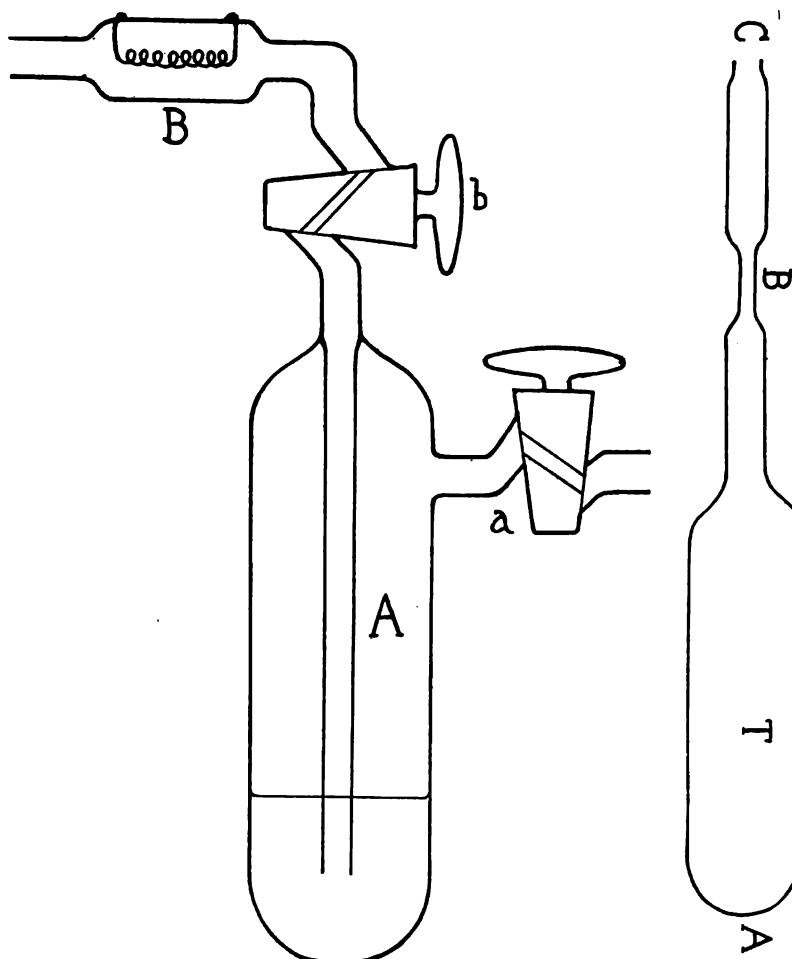


FIG. 1

FIG. 2

through the solution in *A*, we find that light reappears. I think this is conclusive evidence that for *Cypridina* luminescence oxygen is necessary and that the photogenic reaction is an oxidation. The same thing can be demonstrated by other methods.

One of the best ways of removing oxygen from a solution is to add living cells to that solution and allow them to withdraw any oxygen that may be present. If a dense suspension of washed yeast cells is added to a glowing mixture of luciferin and luciferase in a test tube, the luminescence, which previously was uniform throughout the tube, very quickly disappears except at the surface in contact with air. If the tube is now stoppered and inverted so as to allow a bubble of air to rise through the solution, the luminescence reappears but again disappears when the oxygen dissolved from this bubble is utilized. The alternate appearance of light on admitting air and disappearance when the yeast uses up all the oxygen can be observed any number of times. The same thing can be shown by allowing *Cypridina* material itself to use up the oxygen. Other phenomena which indicate that *Cypridina* luminescence is an oxidation will be found in my recent paper (7).

Having demonstrated that oxygen is necessary for luminescence I will now describe the experiment which indicates that all the oxygen cannot be removed from dried powdered (in a mortar) *Cypridina* material with an air pump. Kanda assumes that this can be done and I think his principal source of error lies in this assumption. It is known that when water vapor is allowed to freeze at a temperature of solid carbon dioxide, the ice crystals contain no dissolved gases. We may make use of this fact to obtain a small amount of gas-free water. A glass tube, *T*, of the form shown in figure 2, is surrounded at the closed end, *A*, with solid carbon dioxide in a Dewar tube and kept at this low temperature throughout the experimental procedure. A narrow glass tube is then inserted within the larger tube and moist air directed against the cooled surface, *A*. A layer of frost immediately forms. Care must be taken that drops of water do not fall upon surface *A* and immediately freeze as under these conditions bubbles of gas may be mechanically held in the ice. The narrow tube is removed and a little dried *Cypridina* powder is now placed in the bottom of *T*. By inserting again a narrow tube connected with a hydrogen tank, the tube *T* is filled with hydrogen. The *C* end of the tube must be held down in order that the light hydrogen may not escape. Having filled *T* with hydrogen, it is connected to an air pump, thoroughly evacuated and sealed off at *B*. All this time the *A* end of the tube has been kept at a temperature of solid carbon dioxide. The hydrogen used to fill *T* contained 0.43 per cent of oxygen, let us say 0.5 per cent. The vacuum pump was capable of giving a vacuum of less than 0.5 mm. Hg. The partial pressure of oxygen from the gas left in this

tube after evacuation was therefore less than  $\frac{1}{200}$  of  $0.5 = 0.0025$  mm. Hg. We shall return to this figure shortly.

After sealing, tube *T* is removed from the Dewar tube and the ice allowed to melt. As the water surrounds the dried *Cypridina* powder a fair luminescence results. If the tube is now broken and air allowed to rush in, a perfectly brilliant luminescence results. How can this brilliant luminescence be the result of anything else than the oxygen of the air? Since I have previously shown that when the oxygen is completely removed from luciferin and luciferase no luminescence is visible, how can the first weaker luminescence, which is observed when the ice is allowed to melt, be the result of anything but oxygen which does not come out of the tissue in a vacuum, or the oxygen of the residual gas? It must be admitted that 0.0025 mm. Hg. oxygen pressure is a very low one to cause any kind of luminescence and that the light observed must have been the result of oxygen entangled in the tissue rather than the 0.0025 mm. of the residual gas. I find that 0.0025 mm. Hg. oxygen pressure is far too low to cause phosphorus to luminesce. An experiment performed in exactly the same manner as above but substituting a fragment of phosphorus in place of *Cypridina* powder gave no luminescence whatsoever when the ice was melted<sup>1</sup> but a bright luminescence when the tube was broken and air allowed to rush in.

I report this experiment to show that Kanda's method is not suitable and that oxygen comes from somewhere when dried *Cypridinae* are moistened with gas-free water, even under the most favorable conditions for excluding oxygen, and that the oxygen presumably comes from the tissue itself. We may, however, use the tubes prepared as above to again show the dependence of luminescence on oxidation. If such a tube is not opened immediately, the luminescence continues for a while gradually becoming fainter and fainter. With small oxygen pressure the photogenic reaction proceeds very slowly. In the course of 48 hours the light disappears entirely. The luciferin has used up the last traces of oxygen present. If the tube is now broken and air allowed to enter, a brilliant luminescence results. Tubes containing luciferase, luciferin and water may be sealed in absence of oxygen and kept for months without a trace of luminescence, but if air is admitted a brilliant luminescence immediately appears.

<sup>1</sup> It must be pointed out that phosphorus does not require water to luminesce. The water was merely added to carry out the experiment in exactly the same way as the *Cypridina* powder experiment.

Kanda finds that when he admits water in atmospheres of various gases to his dried *Cypridina* material in bottles, E, E<sub>1</sub>, the luminescence is at first about equally bright with H<sub>2</sub>, N<sub>2</sub>, CO, CO<sub>2</sub>, air and O<sub>2</sub>. Very soon the brightness falls off rapidly in O<sub>2</sub>, fairly rapidly in air, but the luminescence continues bright for a long time in H<sub>2</sub> and N<sub>2</sub>. We may omit the results with CO and CO<sub>2</sub> since these were found to make the solution acid (1, p. 571) and this introduces another factor. Since the bright light lasts longer, the less oxygen is present Kanda concludes "that oxygen is not necessary for the production of light by the material," and "if the production of light by the animal is due to an oxidation, as Harvey claims, the more intense light should be produced by the greater concentration of oxygen. This is not the case, and the writer therefore concludes that the production of light by the animal is not an oxidation" (1, p. 571).

What has happened in Kanda's experiment is this. The initial amount of oxygen present in bottles, E, E<sub>1</sub>, when water is allowed to flow on the *Cypridina* material, is above the amount necessary to give the maximum light. Hence the luminescence is equally bright at first in all gases. In air or oxygen the oxidation goes on sufficiently rapidly to use up the luciferin and reduce its concentration very markedly. Consequently the luminescence fades in these gases. I have found that the intensity of *Cypridina* luminescence is greater the greater the concentration of luciferin, up to a certain limit, and also that the intensity of luminescence, *other factors remaining the same*, does not change when the oxygen concentration is increased from somewhere near 7 per cent to 100 per cent or pure oxygen. In other words, the luminescence intensity is at a maximum when the partial pressure of oxygen is about 53 mm. Hg. (= 7 per cent) and increasing the pressure of oxygen above this value does not increase the intensity of luminescence. The phenomenon is similar to the taking up of oxygen by hemoglobin. At 20°C. hemoglobin is 95 per cent saturated at a partial pressure of oxygen of about 55 mm. Increasing the oxygen pressure above this will not cause much more oxygen to be taken up. As oxyhemoglobin is bright red in color as contrasted with dark red reduced hemoglobin, we may say that it is 95 per cent bright red at 55 mm. pressure of oxygen and will not become an appreciably brighter red if the pressure of oxygen is increased to 760 mm. Hg. (100 per cent or pure oxygen). I have made this comparison with hemoglobin in my book, *The nature of animal light*, now in press and to appear shortly. Many facts bearing on oxidation and luminescence will be found there.



Finally I may add that the experiments here reported were all performed over a year ago, many of them two years ago, but were not published because they demonstrated nothing new. I have recently repeated them to make sure of my facts—and with the same result. I will therefore conclude this article with my statement made in January, 1917 (p. 321), and quoted by Kanda—"Oxygen is necessary for light production as may be seen by placing the crushed animals in an hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears even after a long time, but remains dim so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. Upon readmitting oxygen, however, a brilliant glow results. Every other species of luminous animal investigated likewise requires oxygen for luminescence."

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## A PHYSIOLOGICAL RESPONSE TO PITUITARY ADMINISTRATION

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Notwithstanding the many investigations that have been carried on along the lines of studying the physiological effects of the administration of pituitary substance, there is yet to be found in the literature any report of the changes induced in the amounts of the total nitrogen, non-protein nitrogenous constituents, sugar and alkaline reserve of human blood as the result of its ingestion.

This paper is a report of such a study made on six males, three patients and three nurses at this hospital, none of whom were endocrinopathic. The bloods were taken from any given individual on the same day of the week and but once a week throughout the period of study. The specimens for analysis were taken three and a half hours after breakfast, it having been found that bloods taken at this time are not significantly different in this respect from bloods taken before the morning meal (1).

The total nitrogen was determined by the micro-Kjeldahl method of Folin and Farmer (2) in a 1 to 50 dilution of 1 cc. of the blood. The non-protein nitrogen, urea nitrogen, creatinine, creatine, uric acid and sugar were determined according to the methods of Folin and Wu (3). The estimations of the amino-acid nitrogen and of the alkaline reserve were carried out with the apparatus and methods of Van Slyke and his collaborators (4), (5). The rest nitrogen is the difference between the sum of the nitrogen found as urea, creatinine, creatine, uric acid and amino-acid and the total non-protein nitrogen. As a matter of interest, the twenty-four hour urea excretion was also determined for the days on which the blood was collected. The period of observation for each subject consisted of nine weeks. During the first three weeks no pituitary was fed. During the second three weeks a 2-grain tablet of desiccated pituitary substance was ingested three times a day.

TABLE 1

Table showing the results of the blood analyses before, during and after the ingestion of desiccated pituitary substance

DATE	PATIENTS											REMARKS
	Total N	NP N	Urea N	Kt N	Kn N	UA N	AA N	Rest N	Sugar	Alkaline reserve	Urea N	
	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.		gm.	
9-15-19		44.1	20.5	0.44	1.35	0.60	5.9	15.3	127	55.52	6.3	Subject 1. Pituitary administered from 10-1-19 to 10-22-19
9-22-19	2.82	38.7	19.8	0.60	1.25	0.83	4.5	11.7	92	69.53	14.6	
9-29-19	3.36	42.3	20.0	0.52	1.44	0.77	5.4	14.2	111	69.12	13.8	
10- 6-19	2.73	37.8	18.7	0.44	1.28	0.93	5.3	11.1	94	72.10	10.8	
10-13-19	3.30	42.9	21.4	0.48	1.54	0.80	5.8	12.9	90	60.25	12.9	Subject 2. Pituitary administered from 10-2-19 to 10-23-19
10-20-19	3.33	44.4	24.8	0.48	1.60	1.57	4.2	11.8	115	71.04	10.7	
10-27-19	2.49	40.5	22.2	0.44	1.47	1.10	5.6	9.7	100	65.38	10.7	
11- 3-19	3.00	39.6	20.9	0.48	1.47	1.10	4.5	11.1	100	66.04	10.3	
11-10-19	3.00	48.0	21.4	0.52	1.31	1.03	5.1	18.6	115	71.06	10.9	
9-17-19	3.36	35.7	16.4	0.44	1.31	0.70	4.8	12.0	95	67.88	13.8	
9-24-19	3.06	37.5	15.5	0.52	1.22	0.73	6.1	13.4	119	52.97	10.9	
10- 1-19	3.90	33.0	12.3	0.52	1.35	0.77	4.0	14.1	125	74.80	8.4	
10- 8-19	2.74	34.5	12.4	0.48	1.38	1.07	6.0	13.2	105	69.10	7.8	
10-15-19	3.00	41.7	19.1	0.48	1.35	1.20	6.7	12.9	123	66.62	8.3	
10-22-19	3.33	40.5	16.2	0.48	1.31	1.60	5.9	15.0	93	67.20	8.7	
10-29-19	3.22	45.3	15.1	0.48	1.31	0.87	6.3	21.2	118	71.32	11.0	Subject 3. Pituitary administered from 10-6-19 to 10-20-19 and from 11-3-19 to 11-10-19
11- 5-19	2.85	38.3	15.8	0.48	1.38	1.33	5.8	14.5	105	71.98	9.3	
11-12-19	3.33	39.3	20.3	0.52	1.44	1.16	5.6	10.3	132	71.07	8.0	
9-19-19	3.19	21.0	14.5	0.41	1.38	0.57	4.5		93	63.17	11.7	
9-26-19	3.06	34.2	11.8	0.44	0.96	0.70	3.7	16.6	137	68.60		
10- 3-19	2.83	31.7	17.0	0.44	1.03	0.60	3.9	8.7	95	68.32	7.2	
10-10-19	3.06	34.8	15.0	0.48	1.41	0.67	4.3	12.9	105	65.77	6.7	
10-17-19	2.91	30.3	15.0	0.44	1.22	0.70	4.7	8.2	95	66.20	10.9	
10-24-19	3.00	30.0	16.9	0.44	1.22	0.63	5.9	4.9	105	68.18	6.1	
10-31-19	3.03	31.2	15.7	0.44	1.35	0.70	5.6	7.4	105	63.48	9.3	
11- 7-19	3.00	31.2	17.2	0.52	1.19	0.73	5.3	6.3	95	65.50	11.8	
11-14-19	3.19	32.4	14.6	0.44	1.25	0.67	4.8	10.6	118	61.84	11.9	

TABLE 2

Table showing the results of the blood analyses before, during and after the ingestion of desiccated pituitary substance

DATE	NORMALS											REMARKS
	Total N	NP N	Urea N	Kt N	Kn N	UA N	AA N	Rest N	Sugar	Alkaline reserve	Urea N	
	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.		gm.	
9-28-19	3.12	39.6	13.2	0.48	1.25	1.16	3.7	19.8	103	69.12	9.0	Subject 4. Pituitary administered from 10-11-19 to 11-1-19
10- 3-19	3.18	35.9	15.3	0.48	1.06	0.67	4.1	14.3	85	63.61	7.9	
10-10-19	4.00	32.4	16.3	0.52	1.12	0.70	3.9	9.9	96	65.38	10.2	
10-17-19	2.80	32.4	15.4	0.44	1.19	0.93	4.5	9.9	101	64.75	9.6	
10-24-19	3.30	30.9	17.7	0.44	1.35	0.93	5.4	5.1	111	71.04	6.5	
10-31-19	3.00	31.2	16.3	0.44	1.35	1.20	5.6	6.3	92	63.95	10.6	
11- 7-19	3.15	34.2	13.0	0.48	1.60	1.07	5.8	12.3	82	71.10	7.6	
11-14-19	3.25	36.6	17.3	0.44	1.82	1.10	4.5	11.4	108	63.42	11.4	Subject 5. Pituitary administered from 10-30-19 to 11-20-19
11-21-19	3.06	32.4	16.2	0.44	1.31	1.20	4.5	8.7	119	64.46	10.3	
10-15-19	2.64	35.4	18.4	0.44	1.35	0.90	5.0	9.3	112	63.42	11.0	
10-22-19	3.00	34.5	17.7	0.48	1.31	1.07	5.1	8.8	104	65.30	13.3	
10-29-19	3.00	36.6	13.9	0.44	1.25	1.27	5.7	14.0	126	63.36	10.6	
11- 5-19	3.00	34.3	17.6	0.48	1.47	1.07	4.5	9.2	115	66.24	10.6	
11-12-19	2.82	30.6	14.1	0.48	1.47	0.87	4.6	9.1	118	63.78	8.1	
11-19-19	3.25	32.7	15.7	0.44	1.35	1.10	4.7	9.4	130	62.63	8.8	
11-26-19	3.00	36.9	17.1	0.44	1.66	0.83	5.6	11.3	138	63.54	10.5	Subject 6. Pituitary administered from 9-30-19 to 10-20-19
12- 3-19	2.94	29.0	14.3	0.44	1.38	0.77	5.7	8.4	106	62.55	9.2	
12-10-19	3.30	31.8	12.1	0.48	1.25	0.73	5.1	12.1	113	59.62	15.7	
9-22-19	2.77	33.9	15.1	0.48	0.93	0.80	5.3	11.4	114	61.33	10.0	
9-29-19	3.52	30.0	14.4	0.48	1.16	0.83	4.1	9.2	133	67.22	9.3	
10- 6-19	2.88	32.1	16.6	0.44	1.16	0.87	4.7	8.3	107	63.57	12.8	
10-13-19	3.15	30.6	16.4	0.44	1.38	0.90	4.5	7.1	96	67.55	6.9	
10-20-19	2.94	35.4	16.4	0.44	1.22	1.27	3.9	12.2	132	67.34	10.9	
10-27-19	2.81	37.3	20.1	0.48	1.44	1.00	4.7	9.6	100	67.24	10.8	
11- 3-19	3.00	37.5	17.8	0.48	1.35	1.40	4.6	11.9	105	61.61	10.8	
11-10-19	3.15	32.1	14.3	0.44	1.16	1.03	5.1	10.1	114	63.39	8.7	
11-17-19	3.30	33.3	16.6	0.44	1.41	0.93	5.3	8.6	98	63.54	9.3	

These tables are the results of the determinations of the various constituents and represent amounts per 100 cc. of blood. The values for the nitrogenous compounds are given in terms of nitrogen. The abbreviations NP—non-protein nitrogen; Kt—creatinine; Kn—creatine; UA—uric acid; AA—amino acid; Alk. Res.; Cc. of CO<sub>2</sub> reduced to 0°; 760 mm. bound as bicarbonate in 100 cc. of plasma. The urea nitrogen expressed in grams are the amounts excreted per 24 hours on the day on which the blood was taken.

During the last three weeks the use of pituitary was discontinued. Any variations from this general plan are noted in the tables.

The only valid and consistent change observed is the increase in the blood uric acid occurring with and following the administration of the pituitary substance. This appeared in four out of the six subjects studied. The concentration of this blood constituent did not fall back to the values observed before the period of pituitary feeding during the three weeks following its administration, which may be taken to indicate either a retarded elimination or destruction of the drug, or that its effect tends to outlast its direct action.

Two explanations of the increase in uric acid concentration in the blood are possible, one being that the ingestion of pituitary substance stimulates the nuclear metabolism resulting in an increased uric acid production, the second being that the drug causes a decrease in kidney permeability. A combination of the two effects may be possible. However since Meyers and Fine (6) have shown that as the kidney permeability is lowered the uric acid excretion seems to be retarded to a greater degree than other soluble nitrogenous blood constituents, and since Addis, Barnett and Shevky (7) have found that the subcutaneous injection of pituitrin is followed by a marked depression of the activity of the kidney in the excretion of urea, it would appear as if the increased uric acid of the blood observed as the concomitant and sequence of pituitary ingestion is due to a decreased permeability of the kidneys.

The two subjects not exhibiting this reaction give the physical and mental characteristics of the adrenal type if we accept the classification of Lavastine (8), and while the interpretation of their non-response is purely speculative it is possible to consider that because of this physiological characteristic the administration of pituitary had but little if any effect upon the kidney permeability. This hypothesis is indirectly supported by the findings of Addis, Shevky and Bevier (9) that "the subcutaneous injections of amounts of adrenalin which increase the urea excreting activity of the kidney and of amounts of pituitrin which depress that activity, have no effect when they are injected together in a certain balanced proportion."

There are no differences in reaction to the drug on the part of the normals as distinguishable from the patients.

## SUMMARY

In four out of six individuals studied the ingestion of pituitary substance caused an increase in the uric acid concentration of the blood. This is interpreted as being probably due to a decreased kidney permeability brought about by the administration of the drug.

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## INDEX TO VOLUME LI

- A****CACIA** and associated colloids, action of, 195.
- Acidosis** a criterion of surgical shock, 191.
- Adrenalin.** See *Epinephrin*.
- Adrenalin chloride**, effect of subcutaneous injection of, on heat production, blood pressure and pulse rate, 200.
- Adrenals** and experimental hyperglycemia, 366.
- Air**, dry and moist, effect of breathing, 191.
- Alkali reserve** of blood, lymph and spinal fluid, 551.
- Alpha and beta rays**, physiological effects of, 189.
- American Physiological Society**, Proceedings, 174.
- ANDREWS**, V. L. See **GIVENS**, **ANDREWS** and **McCLUGAGE**, 199.
- Atropine**, antagonism of, to gastrin bodies, 469.
- BACKUS**, P. L. See **COLLIP** and **BACKUS**, 551, 568.
- BALDWIN**, F. M. Susceptible and resistant phases of the dividing sea-urchin egg when subjected to various concentrations of lipoid-soluble substances, especially the higher alcohols, 190.
- BECHT**, F. C. Studies on the cerebrospinal fluid, 1.
- and **P. M. MATILL**. Studies on the cerebrospinal fluid, 126.
- BELT**, A. E. See **HOOPER**, **SMITH**, **BELT** and **WHIPPLE**, 205.
- BERGEIM**, O. See **MILLER**, **FOWLER**, **BERGEIM**, **REHFUSS** and **HAWK**, 332.
- Beta rays**, alpha and, physiological effects of, 189.
- Bile formation, influence of internal secretions on, 193.
- BLEILE**, A. M. and **C. BROOKS**. Further results on the physics of sphygmography, 192.
- Blood, alkali reserve of, 551.
- and circulation in shock, some effects of ether and morphine on, 197.
- cell counts, effect of splenic extract on, 279.
- in clinical shock, 194.
- pressure, effect of adrenalin chloride on, 200, 407.
- —, influence of internal secretions on, 193.
- — observations, capillary, 196.
- —, venous, influence of low oxygen tensions on, 180.
- , rate of oxygen absorption by, 195.
- volume, 205, 221, 232, 257.
- BOOTHBY**, W. M. and **I. SANDIFORD**. The effect of the subcutaneous injection of adrenalin chloride on the heat production, blood pressure and pulse rate in man, 200.
- BROOKS**, C. See **BLEILE** and **BROOKS**, 192.
- BULGER**, H. A. and **P. G. STILES**. The comparative performance of muscles subjected to rhythmic and arrhythmic stimulation, 430.
- BURBAGE**, S. R. See **DRESBACH**, **SUTTON** and **BURBAGE**, 188.
- BURGE**, W. E. The rôle of catalase in the animal organism, 202.
- BURNETT**, T. C. Some remarks on catalase, 184.
- BYRNE**, J. Paradoxical pupil dilatation following afferent path lesion, 188.

- CAMPBELL, JR., W. A.** See **SNYDER** and **CAMPBELL**, 199.
- Capillary blood pressure observations, 196.
- Carbohydrate diet, effect of, on pancreas function, 423.
- Carbon dioxide, physiological action of, 321.
- CARLSON, A. J.** See **JENSEN** and **CARLSON**, 423.
- Cassiopea, effect of oxygen on nerve conduction in, 543.
- Catalase in animal organism, 202.
- , relation of, to heart activity, 182, 525.
- , some remarks on, 184.
- CATTELL, McK.** Some effects of ether and morphine on the blood and circulation in shock, 197.
- Central nervous system, relation of, to epinephrin secretion, 175.
- Cerebrospinal fluid, studies on, 1, 126.
- CHAMBERS, R.** Microdissection studies on the fertilization of the starfish egg, 189.
- CHILLINGWORTH, F. P.** and **R. HOPKINS.** Physiologic changes produced by variations in lung distention. II. Efficiency of the pulmonary circulation in overcoming obstruction, 289.
- Circulation, responses of, to low oxygen tension, 181.
- COLLIP, J. B.** and **P. L. BACKUS.** The alkali reserve of the blood plasma, spinal fluid and lymph, 551.
- — —. The effect of prolonged hyperpnoea on the carbon dioxide combining power of the plasma, the carbon dioxide tension of alveolar air and the excretion of acid and basic phosphate and ammonia by the kidney, 568.
- Colloids, injection of, into circulation, and change in serum refractive index, 257.
- COPE, O. M.** See **LOMBARD** and **COPE**, 174.
- Cypridina, is the luminescence of, an oxidation? 580.
- DANZER, C. S.** See **HOOKE** and **DANZER**, 196.
- DAVIS, N. C.** See **MCQUARRIE** and **DAVIS**, 257.
- DAWSON, A. B., H. M. EVANS** and **G. H. WHIPPLE.** Blood volume studies. III. Behavior of large series of dyes introduced into the circulating blood, 232.
- DOWNS, A. W.** The influence of internal secretions on blood pressure and the formation of bile, 193.
- and **N. B. EDDY.** The influence of splenic extract on the number of corpuscles in the circulating blood, 279.
- DRESBACH, M., J. E. SUTTON, JR.** and **S. R. BURBAGE.** Some observations on dark adaptation of the peripheral retina, 188.
- Duodenum, removal of, 182.
- EDDY, N. B.** See **DOWNS** and **EDDY**, 279.
- Edema, relation of protein deficiency to, 185, 378.
- Electron tube amplification with the string galvanometer, 177.
- Emphysema, chronic pulmonary, pathological physiology of, 178.
- Epinephrin in perfusates of various H-ion concentration, vascular reaction to, 199.
- output, relation of spinal cord to, 484.
- , reactions to subcutaneous injection of, 407.
- secretion, relation of central nervous system to, 175.
- EVANS, H. M.** See **DAWSON**, **EVANS** and **WHIPPLE**, 232.
- EYSTER, J. A. E.** See **MEEK** and **EYSTER**, 180, 303.
- FERTILIZATION** of starfish egg, microdissection studies on, 189.
- Fireflies, flashing interval of, 536.
- FORBES, A.** and **C. THACHER.** Electron tube amplification with the string galvanometer, 177.



FOWLER, H. L. See MILLER, FOWLER, BERGEIM, REHFUSS and HAWK, 332.  
 FRIES, J. A. The respiratory quotient and its uncertainty, 202.

**G**AR roe, chemistry of, 198.

Gastric response to vegetables, 332.

Gastrin studies, 454, 469.

Genito-urinary organs, isolated, action of prostatic extracts on, 203.

GILBERT, N. C. See GREENE and GILBERT, 181.

GIVENS, M. H., V. L. ANDREWS and H. B. MCCLUGAGE. The excretion of a red pigment in the sweat of man, 199.

Glutamine production, effect of, on urinary nitrogen, 202.

GOLDMAN, A. See MUDD, GRANT and GOLDMAN, 184.

GRANT, S. B. See MUDD, GRANT and GOLDMAN, 184.

GREENE, C. W. and N. C. GILBERT. Studies on the responses of the circulation to low oxygen tension. II. The electrocardiogram during extreme oxygen-want, 181.

— and E. E. NELSON. The chemistry of gar roe, 198.

GREISHEIMER, E. See LYON and GREISHEIMER, 191.

GUTHRIE, C. C. See McELLROY and GUTHRIE, 195.

— See WEIL and GUTHRIE, 194.

**H**AGGARD, H. W. See HENDERSON and HAGGARD, 176.

HAMMETT, F. S., C. A. PATTEN and N. SUITSU. A physiological response to pituitary administration, 588.

HARVEY, E. N. Is the luminescence of Cypridina an oxidation? 580.

HAWK, P. B. See MILLER, FOWLER, BERGEIM, REHFUSS and HAWK, 332.

Heart activity, relation of catalase to, 182, 525.

— cycle, time relations of, as shown by carotid pulse, 174.

Heart, mammalian, effects of warming and cooling sino-auricular node in, 201.

Heat production, effect of adrenalin chloride on, 200.

— — — subcutaneous injection of adrenalin on, 407.

— — — in turtle's cardia in response to stimulus through vagus nerve, 183.

Hemato-respiratory function, influence of oxygen deficiency on, 176.

Hemorrhage, circulatory reactions to, 180.

HENDERSON, Y. and H. W. HAGGARD. The influence of oxygen deficiency and related conditions upon the hemato-respiratory functions, 176.

HOOKE, D. R. and C. S. DANZER. Observations on the capillary blood pressure in man with presentation of a new method, 196.

HOOPER, C. W., H. P. SMITH, A. E. BELT and G. H. WHIPPLE. Blood volume studies. I. Experimental control of a dye blood volume method, 205.

HOPKINS, R. See CHILLINGWORTH and HOPKINS, 289.

Hyperglycemia, experimental, adrenals and, 366.

Hyperpnoea, prolonged, effect of, in man, 568.

**I**NTERNAL secretions, influence of, on blood pressure and formation of bile, 193.

IVY, A. C. The rôle of the vagi and splanchnic nerves in the genesis of shock from abdominal operations, 197.

**J**ACOBS, M. H. To what extent are the physiological effects of carbon dioxide due to hydrogen ions? 321.

JENSEN, V. W. and A. J. CARLSON. The apparent influence of a diet of carbohydrates on the pancreas remnant of partially pancreatectomized dogs, 423.

- KEETON, R. W., F. C. KOCH and A. B. LUCKHARDT.** Gastrin studies. III. The response of the stomach mucosa of various animals to gastrin bodies, 454.
- , A. B. LUCKHARDT and F. C. KOCH. Gastrin studies. IV. The response of the stomach mucosa to food and gastrin bodies as influenced by atropine, 469.
- KOCH, F. C. See KEETON, KOCH and LUCKHARDT, 454.
- See KEETON, LUCKHARDT and KOCH, 469.
- KOHMAN, E. A. The experimental production of edema as related to protein deficiency, 185, 378.
- KRUSE, T. Further studies on the action of acacia and associated colloids, 195.
- The interpretation of certain muscle phenomena in terms of "all or none," 182.
- LAMB, A. R.** See NELSON and LAMB, 530.
- LOMBARD, W. P. and O. M. COPE. The time relations of the heart cycle as shown by the carotid pulse of man, 174.
- LUCKHARDT, A. B., M. SHERMAN and W. B. SERBIN. On the origin of the muscular tremors, clonic and tonic spasms in parathyroid tetany, 187.
- See KEETON, KOCH and LUCKHARDT, 454.
- See KEETON, LUCKHARDT and KOCH, 469.
- Luminescence of Cypridina, 580.
- Lung distention, physiologic changes caused by variations in, 289.
- Lungs, circulation in, 289.
- Lymph, alkali reserve of, 551.
- LYON, E. P. and E. GREISHEIMER. Effect of breathing dry and moist air, 191.
- McCLUGAGE, H. B.** See GIVENS, ANDREWS and McCLUGAGE, 199.
- McELROY, W. S. and C. C. GUTHRIE. A method for determining the rate of oxygen absorption by blood, 195.
- McQUARRIE, I. and N. C. DAVIS. Blood volume studies. IV. Blood volume as determined by the change in refractivity of the serum non-protein fraction after injection of certain colloids into the circulation, 257.
- MACHT, D. I. and S. MATSUMOTO. The action of prostatic extracts on isolated genito-urinary organs, 203.
- MANN, F. C. Removal of the duodenum, 182.
- MATILL, P. M. See BECHT and MATILL, 126.
- MATSUMOTO, S. See MACHT and MATSUMOTO, 203.
- MAYOR, A. G. Effect of diminished oxygen upon rate of nerve conduction in *Cassiopea*, 543.
- MEEK, W. J. and J. A. E. EYSTER. Circulatory reactions to hemorrhage, 180.
- — —. Experiments on the pathological physiology of acute phosgene poisoning, 303.
- Microdissection studies, 189.
- MILLER, R. J., H. L. FOWLER, O. BERGEIM, M. E. REHFUSS and P. B. HAWK. The gastric response to foods. VII. The response of the normal human stomach to vegetables prepared in different ways, 332.
- MUDD, S., S. B. GRANT and A. GOLDMAN. Further studies on excitation of infections of the throat, 184.
- Muscle phenomena, interpretation of, in terms of "all or none," 182.
- , work under rhythmic and arrhythmic stimulation, 430.

- NAGAYAMA, T.** Renal activity and the acid base equilibrium, 434.
- The urea excreting activity of the kidney and phosphate excretion, 449.
- NELSON, E. E.** See **GREENE** and **NELSON**, 198.
- NELSON, V. E.** and **A. R. LAMB.** The effect of vitamine deficiency on various species of animals. I. The production of xerophthalmia in the rabbit, 530.
- Nerve conduction in *Cassiopea*, effect of oxygen on, 543.
- Nitrogen, urinary, effect of glutamine production on, 202.
- OXYGEN** absorption by blood, rate of, 195.
- deficiency, influence of, on hemato-respiratory functions, 176.
- , effect of, on nerve conduction in *Cassiopea*, 543.
- tensions, low, influence of, on venous blood pressure, 180.
- Oxygen-want, extreme, electrocardiogram during, 181.
- PANCREAS** function, effect of carbohydrate diet on, 423.
- Parathyroid tetany, muscular tremors, clonic and tonic spasms in, 187.
- PATTEN, C. A.** See **HAMMETT**, **PATTEN** and **SUITSU**, 588.
- Phosgene poisoning, acute, 303.
- Phosphate excretion, urea and, by kidney, 449.
- Physical efficiency tests used by the Royal Air Force of England, 179.
- Pituitary ingestion, physiological response to, 588.
- Prostatic extracts, action of, on isolated genito-urinary organs, 203.
- Protein deficiency and experimental edema, 185, 378.
- Pulse rate, effect of adrenalin chloride on, 500, 407.
- Pupil dilatation, paradoxical, following afferent path lesions, 188.
- RAYMUND, B.** Acidosis a criterion of surgical shock, 191.
- REDFIELD, A. C.** A comparison of the physiological effects of alpha and beta rays, 189.
- REHFUSS, M. E.** See **MILLER**, **FOWLER**, **BERGEIM**, **REHFUSS** and **HAWK**, 332.
- Renal activity and the acid base equilibrium, 434.
- Respiration of dry and moist air, 191.
- Respiratory quotient and its uncertainty, 202.
- Retina, peripheral, dark adaptation of, 188.
- ROGOFF, J. M.** See **STEWART** and **ROGOFF**, 175, 366, 484.
- SANDIFORD, I.** The effect of the subcutaneous injection of adrenalin chlorid on the heat production, blood pressure and pulse rate in man, 407.
- See **BOOTHBY** and **SANDIFORD**, 200.
- SCHLOMOVITZ, B. H.** Further experiments on the effects of warming and cooling the sino-auricular node in the mammalian heart, 201.
- SCHNEIDER, E. C.** Observations on the physical efficiency tests used by the Royal Air Force of England, 179.
- The influence of low oxygen tensions on venous blood pressure in man, 180.
- SCOTT, R. W.** Observations on the pathological physiology of chronic pulmonary emphysema, 178.
- Sea-urchin egg, susceptible and resistant phases of, when subjected to various concentrations of lipid-soluble substances, 190.
- SERBIN, W. B.** See **LUCKHARDT**, **SHERMAN** and **SERBIN**, 187.

- SEYMOUR, R. J. Relation of catalase to heart activity, 182, 525.
- SHERMAN, M. See LUCKHARDT, SHERMAN and SERBIN, 187.
- SHERWIN, C. P., W. WOLF and M. WOLF. Effect of glutamine production on urinary nitrogen, 202.
- Shock, clinical, the blood in, 194.
- from abdominal operations, vagi and splanchnic nerves in, 197.
- , some effects of ether and morphine on blood and circulation in, 197.
- , surgical, acidosis a criterion of, 191.
- Sino-auricular node in mammalian heart, effects of warming and cooling, 201.
- SMITH, H. P. Blood volume studies. II. Repeated determination of blood volume at short intervals by means of the dye method, 221.
- See HOOPER, SMITH, BELT and WHIPPLE, 205.
- Smooth muscle, heat production in, 183.
- SNYDER, A. v't H. See SNYDER and SNYDER, 536.
- SNYDER, C. D. Heat production in turtle's cardia in response to stimulus through vagus nerve, 183.
- and W. A. CAMPBELL, JR. Vascular reaction to epinephrin in perfusates of various H-ion concentration, 199.
- and A. v't H. SNYDER. The flashing interval of fireflies—its temperature coefficient—an explanation of synchronous flashing, 536.
- Sphygmography, results on physics of, 192.
- Spinal cord, relation of, to epinephrin output, 484.
- fluid, alkali reserve of, 551.
- Splanchnic nerves, vagi and, rôle of, in shock from abdominal operations, 197.
- Splenic extract, effect of, on blood cell counts, 279.
- STEWART, G. N. and J. M. ROGOFF. Further observations on the relation of the adrenals to certain experimental hyperglycemias (ether and asphyxia), 366.
- — —. Further observations on the relation of the central nervous system to epinephrin secretion, 175.
- — —. Further observations on the relation of the spinal cord to the spontaneous liberation of epinephrin from the adrenals, and the action of strychnine after cervical cord section, 484.
- STILES, P. G. See BULGER and STILES, 430.
- Stomach mucosa, response of, to atropine, 469.
- — — to gastrin bodies, 454.
- Strychnine, action of, on epinephrin secretion, after cervical cord section, 484.
- SUITSU, N. See HAMMETT, PATTEN and SUITSU, 588.
- SUTTON, JR., J. E. See DRESBACH, SUTTON and BURBAGE, 188.
- Sweat of man, excretion of red pigment in, 199.
- TEMPERATURE** coefficient of flash in firefly, 536.
- Tetany, parathyroid, muscular tremors, clonic and tonic spasms in, 187.
- THACHER, C. See FORBES and THACHER, 177.
- Throat infections, excitation of, 184.
- UREA** and phosphate excretion by kidney, 449.
- VAGI** and splanchnic nerves, rôle of, in shock from abdominal operations, 197.

Vascular reaction to epinephrin in perfusates of various H-ion concentration, 199.

Vegetables, gastric response to, 332.

Vitamine deficiency, effect of, on various species of animals, 530.

**WEIL**, G. C. and C. C. GUTHRIE.  
The blood in clinical shock, 194.

**WHIPPLE**, G. H. See **DAWSON**, **EVANS** and **WHIPPLE**, 232.

**WHIPPLE**, G. H. See **HOOPER**, **SMITH**, **BELT** and **WHIPPLE**, 205.

**WOLF**, M. See **SHERWIN**, **WOLF** and **WOLF**, 202.

**WOLF**, W. See **SHERWIN**, **WOLF** and **WOLF**, 202.

Work under rhythmic and arrhythmic stimulation, 430.

**XEROPHTHALMIA**, production of, in rabbit, 530.



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